Free-Roaming Cheetah Census – South Africa 2022 to 2025

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Abbreviations

Abbreviation	Definition
ACC	Ashia Cheetah Conservation
AWCRC	Alldays Wildlife and Communities Research Centre
AWT	African Wildlife Tracker
BMP	Biodiversity Management Plan
CAG	Cheetah Advisory Group
COT	Cheetah Outreach Trust
DNA	Deoxyribonucleic Acid
ESV	Exact Sequence Variant
FPIC	Free, Prior and Informed Consent
FRCC	Free-Roaming Cheetah Census
FTE	Full time equivalent
FTM	Field team member
GPS	Global Positioning Systems
GSM	Global System for Mobile Communications
IUCN	International Union for the Conservation of Nature
IZW	Leibniz Institute for Zoo and Wildlife Research
NCBI	National Center for Biotechnology Information
NEMBA	National Environmental Management Biodiversity Act
NGO	Non-governmental Organization
NP	National Park
NPAPC	National Problem Animal Policy Committee
PA	Protected Area
PCR	Polymerase Chain Reaction
ppl	People
QDGC	Quarter degree grid cell
RUG	University of Groningen
SANBI	South African National Biodiversity Institute
SMS	Short Message Service
SOP	Standard Operating Procedure
SSR	Simple Sequence Repeat
ST	SpoorTrack
TBD	To be determined
TP	Transfrontier Park
TOPS	Threatened or Protected Species
Vu	Vulnerable

Project summary and abstract

In South Africa, cheetah are managed across four distinct populations: the captive population, the metapopulation in private fenced game reserves and national parks, semi-protected populations in Kruger National Park (KNP) and Kgalagadi Transfrontier Park (KTP), and the free-roaming population persisting on private and commercial farmlands along the borders with Namibia, Botswana, and Zimbabwe [58]. The first free-roaming cheetah population estimates (~600 individuals) in South Africa date back to 2002 [6], relying heavily on regionally isolated population studies with minimal data [79]. Subsequent and more recent estimates lower than ~600 individuals, have likewise depended on modelled densities derived from localised study populations [97]. Given the documented 76% decline in average African wildlife populations between 1970 and 2024 [100], these largely model-based estimates are likely outdated and underscores the urgent need for robust national census data. With national policy documents explicitly stating that "no reliable estimates for the wild population are present" [66], Ashia Cheetah Conservation (ACC, South Africa) initiated the development and coordination of the Free-Roaming Cheetah Census (FRCC; the Census) in 2021, with fieldwork beginning in October 2022. This multi-year initiative (2021-2026) collaborates with Cheetah Outreach Trust (COT, South Africa) and the University of Groningen (RUG, Netherlands).

Of the official IUCN cheetah range in South Africa (~130,000 km^2), **the census focused on ~99,800 km²** of unprotected free-roaming cheetah habitat in the Northern Cape, North West, and Limpopo provinces along the country's northern border. This study area consists primarily of livestock, game and agricultural farming, interspersed with protected unfenced wildlife areas, cities, towns, and large rural settlements [51]. Throughout the fieldwork period, areas of high human densities, fenced game reserves and national parks, geographical barriers such as mountains were systematically validated year by year by the field team to be inaccessible. This left a total area of ~73,200 km^2 in which to specifically execute camera-trapping. Additionally, due to the high population density running through the middle of the IUCN range between Mahikeng in South Africa to Gaborone in Botswana, the assumption was made that this acted as an artificial barrier for natural cheetah movement. For this reason, Northern Cape and most of North West were grouped as **Region 1**, and a smaller portion of North West and Limpopo as **Region 2** (Fig. 3).

The fieldwork during the FRCC involved multiple teams conducting farmer/landowner **interviews** (**N = 299**) for perceived predator/cheetah presence and conflict, land-use, and livelihood impacts. The team also executed systematic **camera-trapping** (**>5.2m images**) for actual cheetah presence and individual identification, genetic sampling through **scat** (**N = 164**) and **blood** collection (**N = 33**), and GPS **collaring** (**N = 20**) to track movements and reveal potential fragmentation barriers.

Population counts were based on standard population estimates, just taking adult individuals older than 24 months into consideration. A total of 119 unique cheetah individuals were identified, **of which only 83 were adult cheetah**, distributed between Region 1 (Northern Cape + North West; N_{Adults} = 33; Table 4) and Region 2 (North West + Limpopo; N_{Adults} = 50; Table 4). The identified individuals reside in a highly fragmented landscape largely caused by differing land use, fencing types, and human population density. Although predators on farmland are often indiscriminately targeted due to perceptions of livestock predation, cheetah are more frequently observed during the day, early morning and early evenings, contributing to them being disproportionally blamed for livestock losses. However, DNA sequencing confirmed that **no livestock** (i.e. cattle, goat, sheep and chicken) **were found in the samples** and at least 52% of the scat samples with prey detected were small game species.

Telemetry data and camera-trap data reflected the cheetahs' activity patterns as well as the **movement differences between the two main study regions**: **Region 1** has a more open, arid landscape with extensive connections to Botswana, while **Region 2** is more constrained and essentially functions as an isolated population with minor movement between Botswana, protected areas in southern Zimbabwe and South Africa, particularly

due to dense human settlement and impermeable (game) fencing. This fragmentation prevents connectivity between the regions which in turn increases isolation and potential inbreeding.

Nonetheless, genetic results are still indicative of a relatively connected population. At this stage, biological fragmentation between the two regions does not appear to be strong **yet**, suggesting that until now or at least recently the gene flow has occurred and the two regions have been genetically connected. However the ecological evidence does point to increasing restriction of movement between the regions, particularly where human population density and impermeable fencing create barriers. Genetic differentiation in long-lived mammal populations, like the cheetah, often becomes detectable only after several generations, typically in the order of 5–15 generations. Given a cheetah generation time of ~4–6 years, this suggests that **measurable genetic structuring may lag behind landscape fragmentation by several decades** [2][33]. **Thus, although full genetic separation has not yet occurred, the conditions are in place for this process to continue if connectivity declines further and population numbers remain below 100 [68].** This provides an important conservation opportunity: if connectivity can be maintained, genetic exchange may support population resilience and reduce long-term isolation risk.

The FRCC findings have direct relevance for national and international policy change. The substantially lower population number identified reflects a combination of improved survey accuracy resulting from the first coordinated cross-country census, as well as ongoing population declines driven by habitat loss, landscape fragmentation and anthropogenic pressures. The Regional Conservation Strategy for Cheetah and Wild Dog in Southern Africa (2015) adjusted the population estimate for free-roaming cheetah in unprotected range in South Africa to ~300 individuals [73]. This new count contributed to the population data utilized by the IUCN Red List Assessment (2021), which estimated ~3526 adult individuals in Southern Africa, retaining its Vulnerable status [26]. In contrast, **the FRCC detected a minimum of 83 adult individuals, representing estimates approximately 72% lower than previously assumed**. Given that the FRCC was the first coordinated multi-year census across free-roaming cheetah range in South Africa, incorporating extensive detection methods across a three-year field period, it is unlikely that a substantial proportion of the population remained undetected. This finding points toward the potential **reclassification of the national free-roaming population to Endangered status under the Regional Red List of South Africa** criteria [29], despite the species not necessarily qualifying as Endangered globally just yet. The study also supports **updating the IUCN range** for southern African cheetah in South Africa. The new range should be based on a combination of confirmed cheetah occurrence, potential suitable habitat, land-use, fragmentation, and social factors affecting the persistence of cheetah in the landscape.

Additionally, the FRCC data are intended to inform the ongoing South African **Biodiversity Management Plan** for cheetah and should be used to strengthen future monitoring and reporting. The report highlights the need for coordinated action across the different cheetah populations in South Africa, including free-roaming cheetah, the semi-protected populations in Kruger NP and Kgalagadi TP, the metapopulation in fenced reserves and the captive population. A more connected national, and eventually southern African regional approach will be essential if South Africa is to maintain viable free-roaming cheetah populations into the future.

Key partner contributions included 1) **Ashia Cheetah Conservation**; primary funder of the FRCC, providing ~70% of the camera traps, 75% of the Global Positioning Systems (GPS) collars, miscellaneous field equipment, continuous in-field staff, management of the timeframe and field coordination. 2) **Cheetah Outreach Trust**; the lead on cheetah capturing, GPS collaring and monitoring. They also provided part of the cameras used in the field and dedicated a significant portion of their time to additional FRCC fieldwork. 3) **University of Groningen**; the PhD student from RUG collected data during an eight-month field period and further analysed all incoming fieldwork data throughout the census. The university provided the funding for the student in the form of a monthly salary. The student acquired additional funding from the 4) **Dr. D.L. Dobberke Foundation** (Dobberke/2229/202225) and 5) the **LNVH Advancing Women in Biology Fund** for travel between countries and a part of the cameras used in the field.

1. Introduction & Context

The Southern African free-roaming cheetah (*Acinonyx jubatus jubatus*) range, modelled by the IUCN to reflect potential suitable habitat based on environmental features rather than confirmed sightings [76], extends across most of southern Africa, crossing Angola, Namibia, Botswana, Zambia, Zimbabwe, Mozambique and South Africa (Fig. 1). This once suitable range is now heavily affected by the growing human population and its accompanying influence on climate and environment, as human settlements and infrastructure encroach upon the cheetah's natural habitat. By 2050, the African human population is expected to have grown to 2.5 billion, a 63% increase of the ~1.55 billion in 2025 [84], suggesting that even further range contraction and loss will be inevitable.

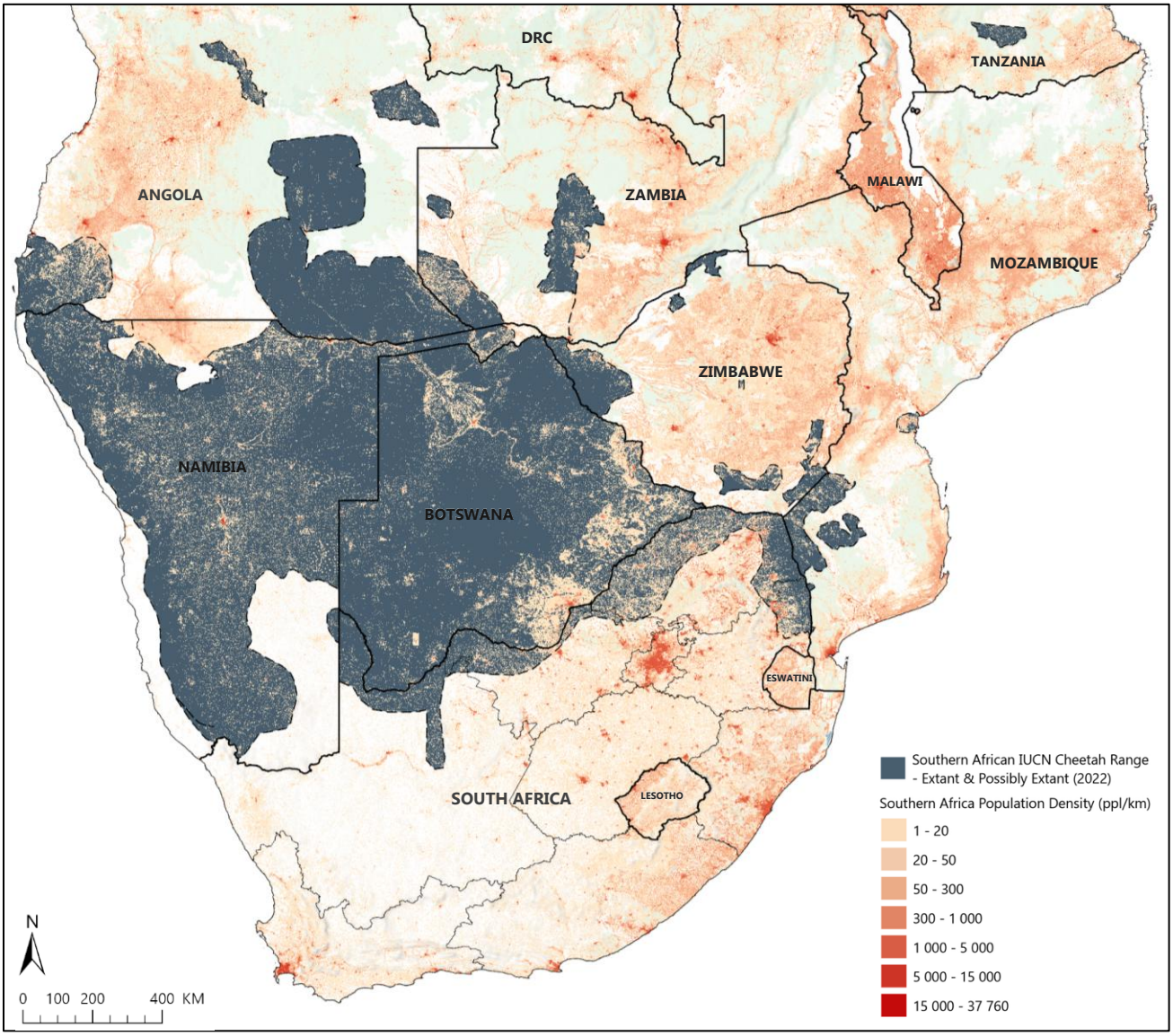

***Fig. 1**: Map of Southern Africa with IUCN A. jubatus jubatus range (2022) with human population density overlap showing the fragmentation and loss of potential cheetah habitat to further human settlement.*

South Africa has the 6th highest population density in Africa, with approximately 63 million inhabitants unevenly spread across nine provinces with higher densities along the coast and in large cities [35]. Northern Cape is the least populated, with an estimated population of 1.4 million people at 4 ppl/km^2, followed by North West with 4.2 million people at 40 ppl/km^2, Mpumalanga has 5.1 million people at 64 ppl/km^2 and Limpopo has 6.4 million people at 50 ppl/km^2 [73].

South Africa's free-roaming cheetah population inhabits one of the most fragmented and human-populated areas of the species' southern African IUCN range, concentrated along the northern border of the Northern Cape, North West and Limpopo provinces (Fig. 2) and consists of approximately 130,000 km² potentially suitable habitat [32][56].

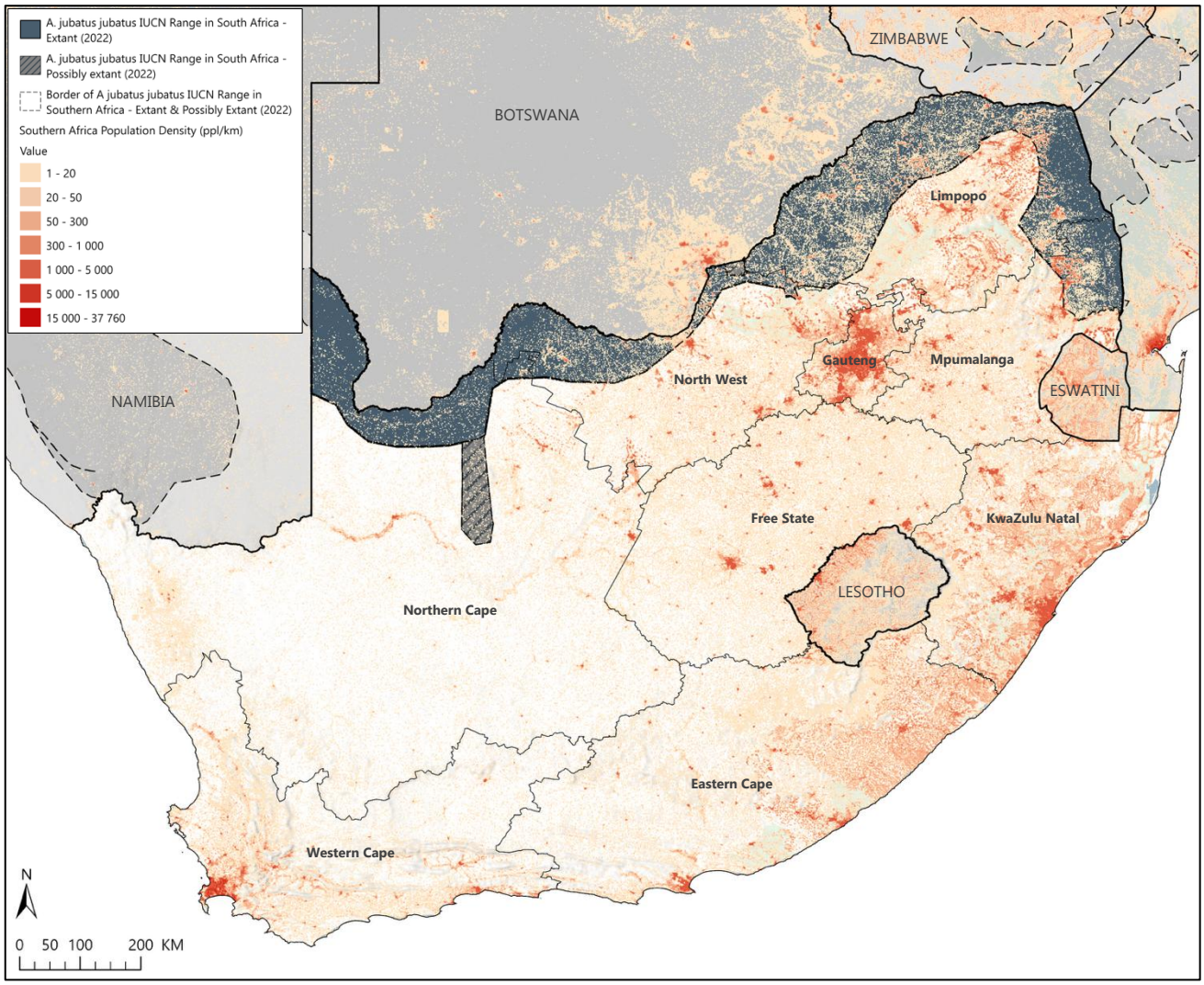

***Fig. 2**: The Southern African IUCN cheetah range with a population density overlay depicting the ongoing fragmentation of previously suitable habitat within South Africa.*

Within this 130,000 km^2 are semi-protected/fenced areas, such as Kgalagadi TP and Kruger NP, fully fenced NPs and private game reserves, urban settlements, towns and cities. Moreover, this range spans a highly fragmented region encompassing ~17,000 farms, forming a mosaic of private and communal lands [28]. Kgalagadi TP and Kruger NP have their own managed cheetah populations [32][13], with low confidence of confirmed natural dispersals in or out of these protected areas (PAs) within South Africa. This makes South Africa a critical area as it holds the southernmost extent of the largest contiguous cheetah range on the continent where fragmentation from various farming practises, (semi-) permeable fencing, and human settlement are main factors of free-roaming population decline [43][69].

1.1 Management challenges

Habitat and land fragmentation restricts animal movement by creating barriers that limits dispersal between populations. This hinders gene flow, increasing the risk of potential inbreeding, and subsequent reduced genetic diversity. Along with limited genetic flow, South African free-roaming cheetah face acute threats from (perceived) livestock/game depredation leading to persecution, road fatalities, and natural prey depletion due

to landscape change [19][74]. This is exacerbated by the country's high human population density (52.8 ppl/km²) and intense farmland fragmentation compared to its surrounding countries like Botswana and Namibia (4.4 ppl/km² and 3.7 ppl/km² respectively; [35]), where vast farmlands enable cheetah movement across larger, less obstructed ranges [8][20][56][94]. With ~75% of South African cheetah range outside fenced PAs, this demographic pressure heightens human-wildlife conflict, demanding targeted management [26][94][53].

Current government conservation strategies and policies lag to maintain and conserve the South African free-roaming cheetah population. Initial calls for a Biodiversity Management Plan (BMP) emerged in 2009 via the South African Action Plan for Cheetah and African Wild Dog Workshop under the National Environmental Management: Biodiversity Act [67], but progress stalled until ACC's 2021 and the Cheetah Advisory Group's (CAG) 2023 efforts, however still no finalised BMP exists. This leaves population genetics neglected, persecution weakly regulated, and potential compensation schemes inadequate, inconsistent or narrowly targeted [3][63][96]. High levels of livestock and game farming make depredation from any predator species one of the most perceived top loss drivers in South Africa, and predatory tolerance is eroded due to financial consequences of high theft, disease and weather pressures and the increase of human infrastructure [60]. This lack of consistent predator management, combined with the ongoing fragmentation of South Africa's landscape, highlights the importance for new, empirical data.

1.2 Knowledge gaps

Reliable data on the current condition of South African free-roaming cheetah is missing. Cited literature is outdated, done on a small-scale or within PAs, and most studies use theoretical models rather than actual observations. Prior estimates of the free-roaming population numbers in South Africa, through theoretical modelling, ranged from 600 individuals in 2002 to 400-800 in 2021 [26][6][97]. The application of theoretical models to management decisions can be constrained by incomplete occurrence data or poor range representation, which hinder accurate species distribution and number estimates and give unreliable predictions when extrapolated across large, unsampled areas [16][54]. Despite this, these early studies did provide invaluable initial insights, highlighting population declines, pinpointing cheetah hotspots, and establishing a moderate understanding of free-roaming distribution and threats in this fragmented landscape. However, consistent landscape transformations, changes in prey availability, human population growth, and the intensification of human-cheetah conflict make it difficult for publications to keep up [14][50], resulting in decision-makers to rely on old figures obtained in only a fraction of the landscape.

The goal of the FRCC was to fill this knowledge gap by gathering directly observed data via landowner surveys, systematic camera trapping, the collection biological samples, and satellite collar tracking within the South African IUCN range. Outcomes include individual identifications for an accurate cheetah count, perceived presence and conflict mapping, genetic analysis for inbreeding, diet analysis to identify favoured prey species, and confirmation of extent of cross-border movement between range countries, replacing assumptions with factual, range-wide data.

1.3 Global implications of the FRCC

By providing updated South African free-roaming cheetah data, the global IUCN assessments can be revised to portray its true state. The cheetah (*A. jubatus*) is classified as Vulnerable (Vu) and current estimates are that southern Africa holds >50% (~3,526 mature individuals) of the remaining global population [26], with South Africa responsible for 10% (~300 mature individuals) within this southern extent [72], but numbers obtained within the FRCC will adjust these estimates substantially downwards. As South Africa makes up the southern range edge, the FRCC informs transboundary connectivity, inbreeding risks resulting in low genetic diversity, and policy like BMPs, influencing Red List updates, funding, and strategies to hopefully slow further global decline of the free-roaming cheetah range (already 91% loss of historic range; [25]).

2. Study area

The aim of the census was to more accurately estimate the South African free-roaming cheetah population size and occurrence outside of fenced/actively monitored PAs within the current IUCN range. Thus the following was excluded from the census: Kgalagadi TP and Kruger NP, the ~13,000 km^2 range in Mpumalanga (no confirmed free-roaming cheetah sightings; human settlement barrier causing separation from the cheetah population in Limpopo), a portion of the possible extant range in the Northern Cape looping southward (historically no free-roaming individuals have been confirmed) and all fenced PAs such as game or nature reserves. Accordingly, of the total IUCN cheetah range in South Africa (~130,000 km^2), the census focused on ~99,800 km^2 of unprotected range.

Due to the high population density running through the middle of the IUCN range between Mahikeng in South Africa to Gaborone in Botswana, the assumption was made that this acted as an artificial barrier for natural cheetah movement. For this reason, Northern Cape and most of North West, characterized predominantly by semi-arid savannah habitat, were grouped as Region 1, and a smaller portion of North West and Limpopo, dominated by grassland and bushveld habitat, were grouped as Region 2 ([51] ;Fig. 3).

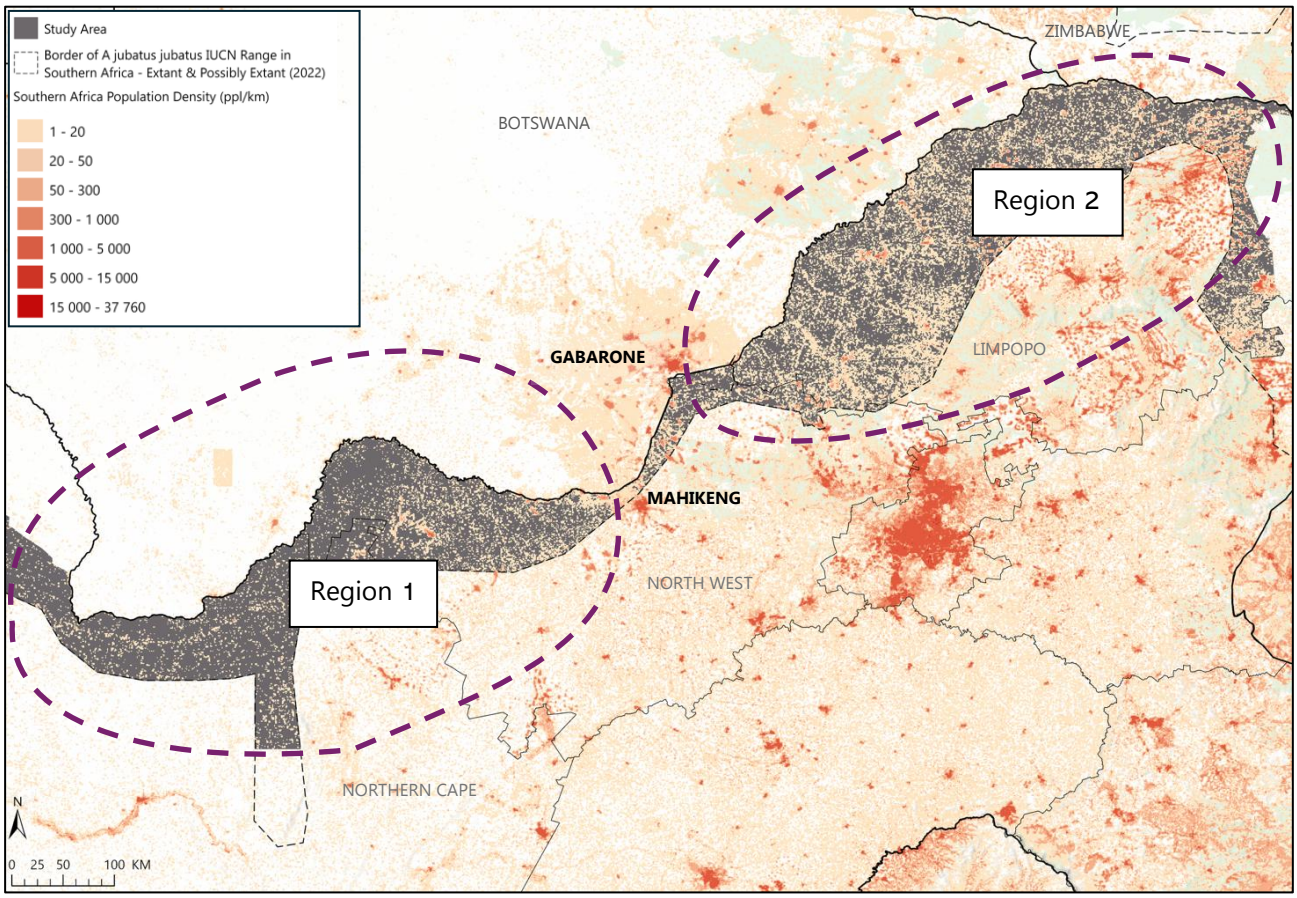

***Fig. 3**: The division of the study area in Region 1 (Northern Cape + North West) and Region 2 (North West + Limpopo) due to the high human population density between Mahikeng (South Africa) and Gaborone (Botswana).*

The census range (~99,800km^2) was overlaid with a quarter-degree grid consisting of ~25 x 25 km cells (QDGC; Fig. 4), which ensured a systematic approach to data collection. Each QDGC had its own name based on province, column and row number (i.e. NW_D4 is in the North West province, in column D, row 4). Within this study area, the landscape consists primarily of livestock, game and agricultural farming, interspersed with protected wildlife areas. Additionally, part of the study area contains cities, towns, and large rural settlements.

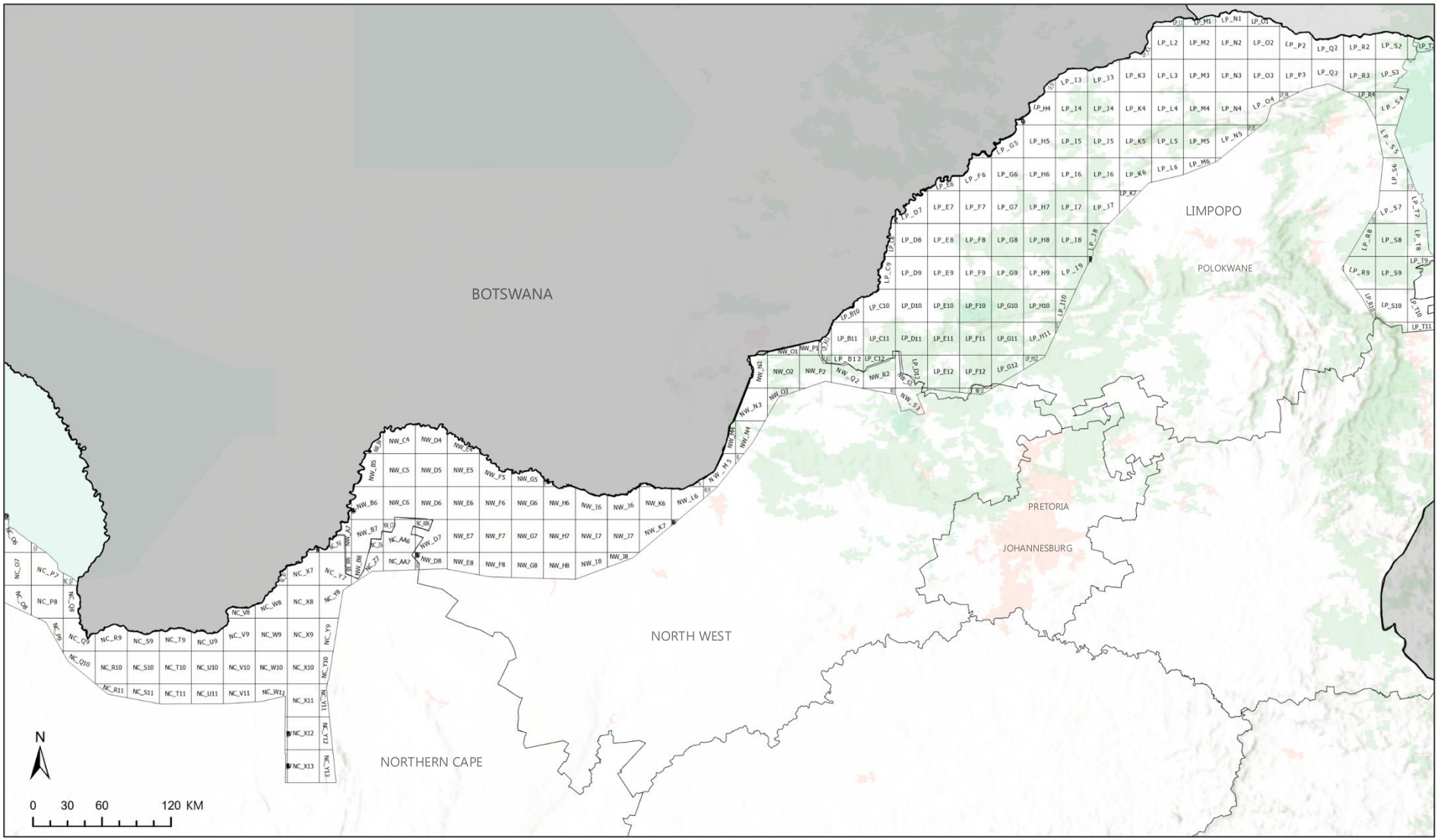

***Fig. 4**: The study area, minus Kgalagadi TP and Kruger NP, with a QDGC overlay for systematic sampling. The area is overlayed by a total of 261 grid cells.*

3. Census methods

The FRCC utilised five survey techniques: questionnaires with landowners/farmers/workers, camera-trapping, biological sample collection (i.e. scat and blood), public sightings and telemetry data from collared individuals.

3.1 Questionnaires

The use of questionnaires was essential for a better understanding of the human attitude towards various species and conservation efforts that camera traps or remote sensing only could not capture. The questionnaire used for the FRCC was developed by the census collaborators in KoboToolbox, a free, online software for collecting and managing data [62], and comprised of 329 questions (Appendix I). Not all questions needed to be answered, as conditional branching (skip logic) questions were used, meaning that many follow-up items were only displayed when participants answered 'yes' to certain questions.

The survey covered a broad range of topics including, but not limited to, general farm and farmer information, land-use practises (agriculture, ecotourism, game and livestock farming), and the presence of and conflict with free-roaming predators. The validity of predator-related responses were analysed with the help of a predator recognition section within the survey (Appendix II). Participants were shown one-to-two randomized full body and fur-only pictures, where they needed to specify the specific predator presented to them.

Questions on livestock/game losses, predator presence and conflict, captured not just self-reported economic costs and species numbers but also emotional and social impacts, revealing tolerance levels critical for predator coexistence on farmlands. Livelihood loss questions quantified predation versus other causes (e.g. disease and theft), while questions related to welfare gave insight into broader hardships like financial strain from lacking adequate fencing or insufficient compensation, informing why farmers tolerate or persecute predators. The combination of perceptive survey data with actual camera trapping and GPS data highlighted biases, such as overestimating cheetah presence despite moderate reports.

3.1.1 Questionnaire spread

The surveys were executed flexibly to maximize participation across the vast study area. Meaning that participants completed them independently via an online KoboToolbox form, with a fieldwork team member physically present, or telephonically. All participants were informed of the study's purpose, procedures, and potential risks, and Free, Prior and Informed Consent (FPIC) was obtained in accordance with established social-science protocols [47]. Respondents could choose to remain anonymous, which promoted honest reporting of sensitive information and reduced bias, particularly regarding controversial practices. The questionnaire spread primarily through word-of-mouth among farmers, amplified by flyers featuring a direct link and QR code to the survey for easy access. To boost appeal, COT offered prizes; extensive toolboxes, an MSC cruise to Mozambique and a tourism package to the Limpopo Lowveld, with winners randomly selected at the end of each census year. Non-anonymous participants received follow-up contact from FRCC team members, inviting them to join an already established WhatsApp group of >200 farmland owners. This platform shared census updates, cheetah sightings, farmer opinions, and contacts. Such engagements built lasting trust and might enhance long-term conservation collaborations.

Questionnaire responses were submitted both inside and outside of the study area. Submissions outside the study area (N = 36) were valuable, as reports of absent or present cheetah or other predator sightings beyond their recognized range provided important information for understanding current perceptions of distribution.

For upcoming questionnaire analysis, only responses within the study area were used (N = 299). Response rates were highest during the autumn and winter months, with numbers going up to 30 surveys in August 2024. The holiday periods resulted in calmer months with (close to) zero responses (Fig. 5).

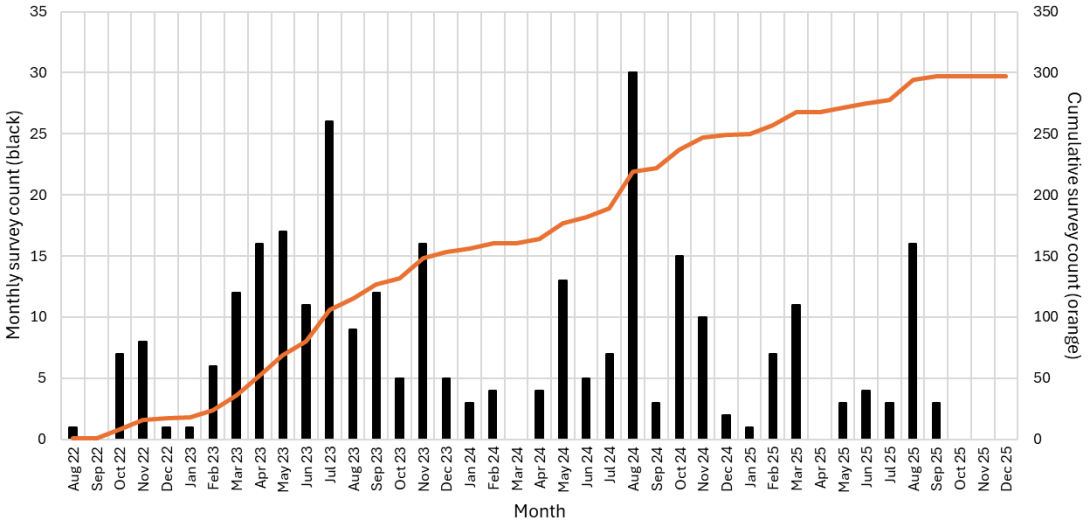

Fig. 5*: The cumulative number of surveys during the study (orange line), going up to 299 surveys, and the number of surveys executed in each month separately (black bars).*

3.1.2 Importance

The FRCC questionnaire data can help inform South African policy about the extent of **perceived** cheetah presence and conflict to advocate expanded compensation schemes, permeable fencing incentives, and wild prey maintenance on game farms to reduce depredation. Beyond metrics, this questionnaire created a focus that was not only ecology, but also human-centred, which is vital in these human-dominated landscapes and builds necessary social connections with the farmers, fostering trust through face-to-face interactions.

3.2 Camera Trapping

Camera trapping was designed to focus on fine-scale cheetah presence, activity patterns and, most importantly, individual cheetah identification for an accurate baseline of the free-roaming cheetah numbers in South Africa. During the initial phase of the census, camera trap placement was strategically guided by farmer reports of cheetah sightings from the landowner questionnaires, which directed us to high-priority areas of confirmed or perceived cheetah presence. This survey-informed camera placement maximized early detection success across fragmented farmlands, before expanding to broader deployment to also confirm cheetah absences. Throughout the fieldwork period, areas of high human densities (i.e., informal settlements, towns, cities), fenced game reserves and national parks, geographical barriers such as mountains were systematically validated year by year by the field team to be inaccessible for camera-trapping. **This left a total area of ~73,200 km² accessible to specifically execute camera-trapping.**

3.2.1 Preparation

Four quadrants (~12.5 x 12.5 km) were established within each QDGC and used to ensure camera sites were not spaced too far apart, while optimizing limited cameras and resources across the vast study area (Fig. 4). This spacing kept the distances between cameras well within free-roaming cheetah home range sizes, ensuring all individuals had a chance of being photographed at least once. Specifically, single male home ranges could span from 494 to 663 km² (~24 × 24 km), coalitions could go up to 849 km² (~29 × 29 km), and females could range from 241 to 306 km² (~17 × 17 km) [85][70][42]. Quadrants of 12.5 × 12.5 km thus fell within these ranges, increasing the chance of individual capture.

Before going into the field, each camera received a unique, permanent 3-digit label on both the camera itself and the inserted SD card according to the Standard Operating Procedure (SOP; Appendix III).

3.2.2 Sentinel sites

Within each QDGC, potential sentinel sites were identified across the landscape to guide camera trap deployment. These sites may act as communication hubs and therefore have a high likelihood of having cheetah present. Sites were confirmed to be potential communication hubs with cheetah presence from either physical sightings or surveys and the identification of scent marking such as spoor, scat or scratching. Alternatively, areas where cheetah passage seemed most probable due to landscape features like fence gaps or waterholes, were also marked as potential camera deployment options [27][89].

Marking/play trees were the preferred sentinel sites for camera placement, and potential marking trees could be identified by climbable, almost horizontal branches offering vantage points for cheetah [27][55]. Favoured tree species within our study area were the Shephard's/Witgat and Marula tree as these trees often have low-hanging, sturdy branches, allowing cheetah to climb (Fig. 6). Thereafter, fence holes were prioritized if there was a clear animal pathway with predator signs present or, in the best-case scenario, cheetah spoor/fur evidence. Fixed water sources (boreholes, pumps, troughs) provided a final option. These places are not often visited by cheetah due to their low water dependency [89], but they do attract multiple (predatory) species and are used as areas of communication.

Fig. 6*: Four different Shephard's trees, each with their own potential cheetah sign present. Left to right: spoor, scratches, possible kill and scat.*

Even in quadrants where no cheetah sightings were reported, cameras were deployed at possible sentinel sites to confirm absence rather than assuming it from perceptions alone. This approach provided empirical evidence of cheetah distribution limits, distinguishing true absence from perceived absence due to under-reporting or tolerance biases in survey data. For a full grid cell (~25 × 25 km, unaffected by borders) to be completed, the aim was to have a minimum of **three** permanent camera sites, each with active camera pairs, distributed across at least three quadrants (Fig. 7). The maximum number of sites were left up to the field staff, as cameras could be moved around the grid to increase capture potential. Sites received unique names combining province code (NC for Northern Cape, NW for North West, and LP for Limpopo), grid identifier (column letter + row number), and a number restarting at 001 per province-grid combination. For example, the

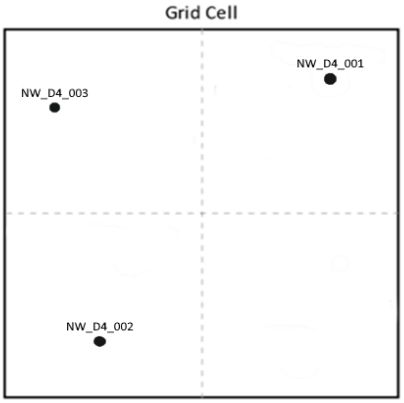

Fig. 7*: The identification and naming of sentinel sites in a grid cell divided into four quadrants (dotted lines).*

first site identified in grid D4 in the North West province will be called NW_D4_001. Multiple "001" sites could exist across provinces but not within the same grid cell. Cameras could be deployed immediately upon site identification or later on, but the sentinel site number remained fixed to its original location, even during inactive periods without cameras.

3.2.3 Camera placement

The placement of cameras was done in pairs, where two cameras would face the sentinel site in a north-south direction to minimize sun glare during sunset and sunrise, but they would never directly face each other to prevent triggering and blinding the opposite camera with the flash (Fig. 8A). By using two cameras, both the left and right flanks of passing cheetah could be photographed, which was crucial for individual identification based on unique spot patterns (Fig. 8B). In the case of too much uncertainty about the success rate of a newly chosen sentinel site, the site would start of as an unpaired 'recce' site. These sites would only have one camera active for a short period of time to scout the area for wildlife activity. These temporary setups quickly assessed whether a potential sentinel site was suitable to become a paired 'permanent' site for cheetah identification before using too many resources.

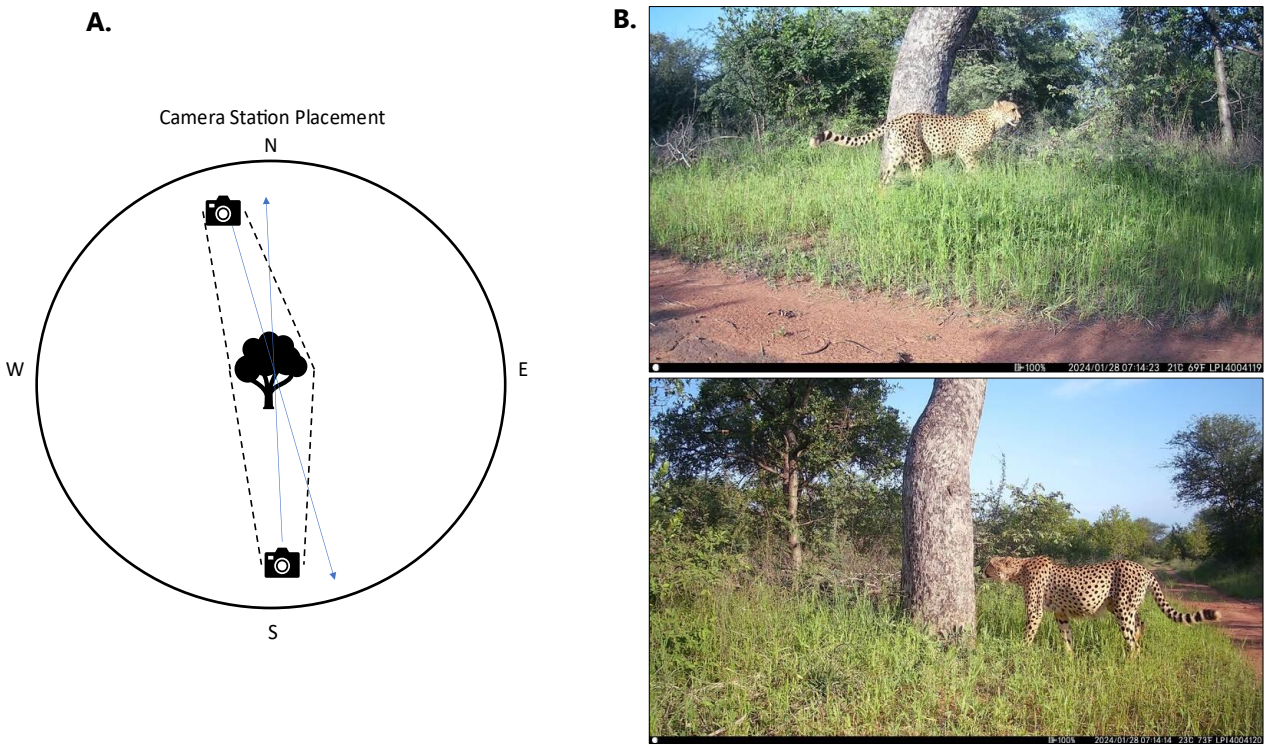

***Fig. 8**: **A.** Camera trap placement at a play tree in north-south directions, while avoiding direct flash triggers. **B.** The imagery of both left and right flanks of a single individual at the same time for correct identification.*

The distance of the cameras from the sentinel site was on average between 5–8 m. Once optimal camera positions at a site were identified, features in the landscape such as fence lines and trees were used for mounting cameras. If this was not available, rough, triangular steel droppers/poles (used for a better grip in the soil compared to smooth, round poles) were utilised and hammered ~20 cm into the ground, extending ~1.80 m above the surface. Cameras were secured inside protective metal cages (when available) and mounted with cable ties/wire at a height of ~1.60 m. This height was optimal for capturing full flank profiles (ideal for cheetah identification) while deterring damage from most wildlife. Before activation, sites were cleared of long grass, overhanging branches, loose twigs and rocks to minimize false triggers. Cameras were configured consistently, with the date and time set to DD/MM/YY HH:MM format. The cameras were set to high trigger sensitivity with 5-second intervals. This sensitivity could be changed lower or higher if needed (e.g. when the site produced

many false triggers, or no images at all). The temperature was set to Celsius, sounds of the camera were disabled, and the overwrite function was turned off to prevent any loss of images.

After camera activation a 'reference sheet' was held up in front of both cameras that provided information on what action was done (camera activation or check), farm name, site ID, date/time and camera numbers. This provided timestamp recovery if cameras reset to default (often to 01-01-2020) from animal impacts or falls and, to factor in human error and location recovery if image folders were labelled incorrectly. When a site was activated for the first time, the aim was that the first site check was executed 48 hours after set-up to confirm if the positioning and photo angles were correct.

After this, preferably 6-8 week servicing intervals for battery/SD card/site maintenance were used, where images of a site were uploaded onto a hard drive in a site-specific folder. These folders were labelled by province, grid, site, camera and the date between which the images were taken (e.g. NW_D4_001 Cam_023 01/01/2023 – 15/02/2023). After emptying the SD card and replacing the batteries, the cameras would get reactivated. Reactivations used identical reference sheets as the initial activation. After each check farmers received ≥1 photo per detected species per visit with full image reviews available on request. At times, access to camera sites to meet the 6-8 week servicing window were limited due to heavy rains, unavailable landowners or other logistical complications.

All activations, checks, and retrievals were systematically logged via an online site operations KoboToolbox form created by the census' collaborators, which recorded the date of site identification, location, type of sentinel site including an image, installation dates, placed cameras, and subsequent checks and removals of the cameras (Appendix IV). Subsequently, this information was relayed to an online ArcGIS map for field staff to refer to. By the end of the census, a total of 389 cameras of 11 different models were deployed (Table 1; Appendix V).

***Table 1**: The different camera trap models used for the FRCC.*

	Camera Model	*N*
1	Browning BTC-5HDX	7
2	Bushnell 5710	3
3	Bushnell 119932C	4
4	Bushnell 119936C	6
5	Cuddeback 1279	58
6	Panthera V7	31
7	Rik Rhino BG583	3
8	Solar Ceyomur CY95	60
9	Solaris Weapon 4K	20
10	Solar Wifi	25
11	Spartan SR3-CX	139
12	Spartan SR4-BK	32
13	Vista Trail	1
		389

While camera sites were primarily retrieved after a grid cell was considered complete (i.e. three camera sites across three quadrants for a period of ≥100 days), additional retrievals could occur when a site showed low camera safety. For example, sites were deactivated if one or both cameras were stolen to prevent any further losses, or when certain wildlife presence causes damages by knocking down or rubbing against cameras. Combined with weather damage and a handful of faulty units, these factors resulted in 106 damaged cameras (27%) and 27 stolen (7%).

3.2.4 Importance

Knowing the baseline number of free-roaming cheetah in South Africa is critical for effective conservation planning, as an estimated 25% of the southern African population resides here [25]. Previous estimates from Buk *et al.,* (2017; [13]) and Weise *et al.,* (2017; [97]) relied either on small-scale studies that were extrapolated across vast landscapes or the use of model estimations, often overestimating actual numbers. The FRCC's grid-based, landscape-scale approach provides the first robust cheetah numbers and distribution on these fragmented farmlands, avoiding extrapolation biases and delivering precise data for IUCN assessments.

3.3 Public sightings

Beyond systematic camera traps and questionnaires, public cheetah sightings were actively collected. When visiting the farmers for questionnaires or camera work, the field team invited them to join a dedicated WhatsApp group (>200 members across the study area) created by COT prior to the census and served as a real-time platform for sharing cheetah sightings, suspected predation, spoor and scat. The team provided participants of this group with census updates via infographics, and any questions with regards to the work, both field and desktop, were answered. Farmers typically reported sightings via this WhatsApp group with GPS coordinates and accompanying photos or videos when available, specifying cheetah numbers and sometimes even sex and age. If preferred, farmers could also message field team members privately. Troublesome or large groups of cheetah that were reported that needed urgent reaction, would fall under COT purview for potential conflict mitigation options and/or capture and satellite collaring.

Due to inherent reporting bias, public sightings without image evidence always required cautious interpretation when physical follow-up was infeasible. Farmers with low tolerance for cheetah were more likely to report potential sightings and predation, which could shift the blame from a more likely predator, such as leopard (*Panthera pardus*) or black-backed jackal (*Canis mesomelas*), creating inflated cheetah presence estimates. This tolerance-driven bias underscores why it was important to have sightings cross-validated with camera trap data, survey predator recognition scores, and/or photographic evidence, to ensure reliability. Nevertheless, this open contact system extended monitoring reach beyond physical visits, while building community trust and engagement.

3.4 Telemetry

Tracking data gained from satellite collared cheetah provided complete movement data that were used to assess transverse patterns across farmlands, and determining landscape connectivity, providing a complimenting addition to the static camera trap data. In an ongoing effort of conflict mitigation, COT trapped, collared, and at times relocated, in coordination with provincial authorities, suspected damage-causing cheetah to prevent deadly retaliation by farmers.

3.4.1 Cheetah capture

Various capture methods consisting of free-darting from a helicopter or from the ground if the individuals' location is known, to capture cages at sentinel sites, were utilised. When the decision was made to trap, a suitable sentinel tree was selected and a temporary "fence" of branches and dense thorn bushes constructed around it, creating a single access point to the scent-marking site (Fig. 9). This is where the trap was positioned, sometimes baited with scat from other cheetah sourced from field collections or facilities.

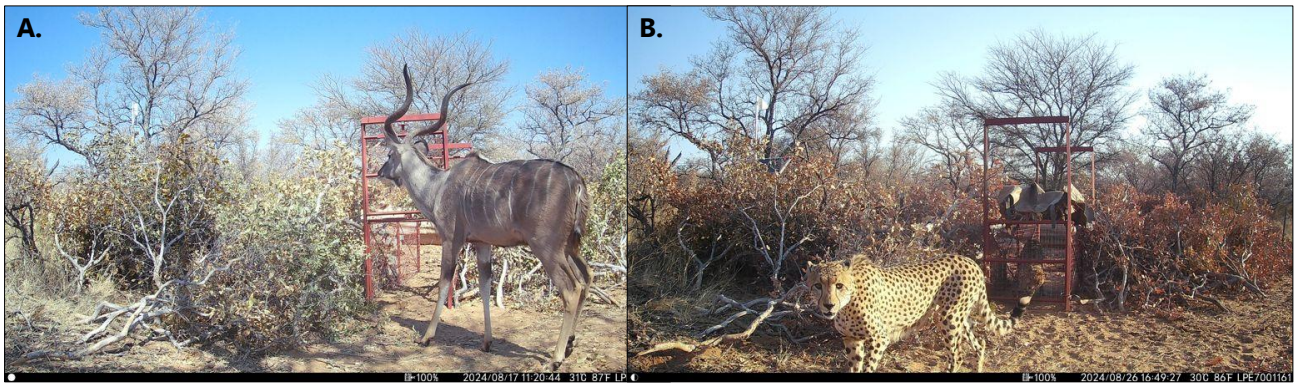

***Fig. 9**: A trap cage set-up at site LP_E7_001 (Region 2). **A.** Open cage surrounded by branches leaving the cage itself as clear path for cheetah to use. **B.** A cheetah individual has triggered the cage mechanism and is captured, his coalition mate close by.*

Two different trap cage systems were used during the census: 1) mechanical trigger cages with an open trap door on either side with a pressure plate. When the animal steps on the plate, both doors drop simultaneously. These trap cages were monitored daily to see if a cheetah had been captured. 2) Solar powered magnetic sensor trap cages that closes upon animal entry as sensors are triggered, simultaneously notifying field staff via a GSM Commander Unit (a monitoring system sending real-time SMS or App alerts). This type of trap cage is ideal when daily checks were impractical and was designed and first utilised in Namibia during another free-roaming cheetah project by Leibniz-IZW, coordinated by J. Melzheimer [81].

Once a cheetah had been trapped, it would either be sedated while still inside the capture cage or alternatively be channelled into a transport crate and transported to a temporary holding facility pending release at the capture site or nearby. During sedation both the left and right flank of the individual were photographed for individual identification. Biological samples were collected for analysis to provide prey preference, relatedness and assess inbreeding in the free-roaming population (sections 3.5, 3.6). The date of capture, number of individuals captured and collared, the sex, estimated age, and collar numbers were recorded in KoboToolbox (Appendix IV).

Lastly, a SpoorTrack (ST) or Africa Wildlife Tracking (AWT) satellite collar was fitted, leaving a two-finger width gap between collar and neck to prevent the collar from slipping into the mouth but loose enough to avoid constriction or choking. The ST collars have rechargeable solar batteries and could last the potential lifespan of a cheetah (averaging to eight years in the wild, [49]). The AWT collars often last for approximately 5,000 location pings (or ~1.5 years) before the battery is depleted. Because of this, AWT collars had drop-off units attached, which auto-release after a set date (~1.5 years from activation), transmitting a final location for recovery. Both collar types send location updates every 4–6 hours (customisable based on needs). If signals were not transmitting properly, not transmitting at all or showed suspicious behaviour (i.e., static next to a road) a field team member would visit the area to investigate the last confirmed location. In return for allowing trapping of cheetah on their land, COT occasionally shared the GPS locations with the farmers who would use this information to predict cheetah movements and safely move their livestock and/or game to a different kraal.

3.4.2 Importance

The use of GPS collars revealed how cheetah moved across the area and showed some cheetah mortality and its rate, giving real-time insights as an addition to the static data of surveys and camera traps.

3.5 Scat

Cheetah roam farmlands holding a variety of animal stock and have long been the scapegoat for small-sized livestock losses on top of the expected game losses experienced in these remote areas. By collecting suspected cheetah scat samples, these claims were tested. Scat analysis offered two key insights: host identification and prey identification. Hair found in the scat were compared to reference hair databases to match samples to specific prey species [88][1][11], and DNA sequencing confirmed both the prey and host species [91][87]. This ultimately revealed whether cheetah predominantly preyed on (wild) game, livestock, or a mix of both. Furthermore, cheetah DNA from scat were linked to genetic profiles, tying diet to specific areas.

3.5.1 Collection

Scat was collected opportunistically during field work. Cheetah scat was recognized by its relatively short sections and the absence of white, calcium-rich material (Fig. 10), typical of hyaena or leopard scat, and often found on or around sentinel trees or prominent bushes. Once located, each scat sample was collected in a brown paper envelope, receiving a standardized sample code starting with "S" (e.g. S_001), followed by farm name, precise coordinates, scat condition (soft, hairy, hard), date/time, and the confidence scale (1-5) that it was cheetah. This same information, with an option to add an image of the scat, was uploaded in an online KoboToolbox form (Appendix IV). Only a portion of the scat was sampled to maintain the individual's scent at the site, encouraging cheetah to continue using the area. The collected samples were kept out of direct sunlight until transferred to ACC for storage.

***Fig. 10**: Scat found on the trunk of a Witgat tree (A and B), and cheetah scat found in a small bush on the ground (C).*

Despite frequent cheetah detections on camera traps at certain sentinel sites, scat could not always be found during site checks. The lack of scat at these sites could be explained by seasonal rainstorms in the northern provinces disintegrating and washing away possible scat before collection, other predators having eaten the scat (coprophagia), telecoprid behaviour of beetles (sculpting and rolling balls of scat away; [22]), or the cheetah could just be passing through without defecating. This mismatch between cheetah captures on camera versus cheetah scat highlights why camera trapping and scat collection complement rather than substitute each other.

3.5.2 Importance

Cheetah scat collection and subsequent analyses directly addressed farmer perceptions of livestock/game predation by providing diet evidence, revealing prey preferences. This evidence can boost farmer tolerance by demonstrating low livestock depredation rates, supporting targeted interventions like wild prey enhancement on game farms. These results could educate farmland owners and reframe cheetah as ecosystem regulators in the form of top-down control, meaning that cheetah selectively target weaker, possibly diseased animals, preventing pathogen spread and maintaining herd health [98].

3.6 Genetics

Genetic sampling was opportunistically incorporated into the census as a complimentary component. While not the central focus, it did provide an additional layer of information that enhanced the understanding of the population structure and connectivity between the two regions.

Given that this type of sampling is uncertain in terms of sample quantity and quality, an integrated and varied approach was undertaken. Microsatellite analysis, also called Simple Sequence Repeat (SSR) genotyping, was employed for all genetic samples collected. Microsatellites are highly variable, meaning that the number of times a short DNA sequence is repeated in tandem at a specific genomic location differs between individuals within a population, and thus provides a high-resolution tool for distinguishing between individuals. It also differentiates between homozygous (inheritance of the same allele from both parents, e.g., AA or aa) and heterozygous (inheritance of two different versions of an allele, e.g., Aa) markers. Identifying heterozygous individuals is important as it shows that an individual carries two different versions of a gene at a locus, which is one sign of genetic variation within the population. The more heterozygous individuals are present in a population, the more genetic diversity the population is likely to have overall.

3.6.1 Scat samples

Non-invasive DNA was extracted from scat samples following standard laboratory protocols at the Jonah Ventures (laboratory) in Boulder, Colorado, USA and split between host DNA and prey DNA. The confirmed cheetah host DNA were analysed by the University of California Davis Veterinary Genetics Laboratory in Davis, California, USA., for individual identification and familial relationships.

3.6.2 Blood/Skin samples

Samples were collected during collaring and sedation procedures or opportunistically from deceased animals. In addition, archived samples from the SANBI Biobank were incorporated to expand the dataset. The inclusion of these samples was important for comparative purposes, improving confidence in individual identification and the broader population-level interpretations. All collected samples were analysed by the Unistel Medical Laboratory in Cape Town, South Africa.

The dual lab approach was largely due to logistical and regulatory complexities associated with international sample export, particularly the permitting requirements under CITES. Despite these constraints, the inclusion of genetic data aimed to strengthen the overall FRCC dataset and provide valuable, albeit supplementary, insights to the remaining population.

4. Results

4.1 Farm land-use

Responses occurred within 147 grid cells, covering ~80,900 km^2, of which ~59,000 km^2 (73%) is inside South Africa's IUCN cheetah range. A total of 299 farmers within this range filled in their responses (Table 2), which is considered sufficient for covering our study area based on a published and internationally recognised cheetah presence study by Van der Meer (2018; [93]), where 1,209 people were interviewed across Zimbabwe which has a size of 399,757 km^2. This indicates that within the FRCC study area, of size 99,800 km^2 (section 2), 302 surveys are needed for similar coverage. With 299 respondents, the census was on target for the needed amount to match the coverage of Van der Meer.

***Table 2**: Census effort resulted in a total of 335 executed questionnaires, of which 299 (89%) were within the study area.*

	Inside IUCN range	Outside IUCN range	Total questionnaires	Total anonymous
Region 1	141	33	174	20
Region 2	158	3	161	29
	299	**36**	**335**	***49***

This section summarises the results of the survey questions relating to land-use practices across the study area. The land-use categories identified include agriculture, livestock farming, game farming, and ecotourism, where participants were able to reply with multiple land-use practices if applicable. For livestock and game farmers, further distinctions were made between different species to provide a more detailed understanding of land utilisation and how both domesticated and wild species occupy the landscape.

To spatially represent these data, each grid cell was subdivided into four quadrants, similarly to the camera trapping methodology. Each quadrant was assigned a land-use category based on the proportional representation of questionnaire responses within that grid cell. For example, if four questionnaires were conducted within a grid cell and two responses reported agriculture, one reported game farming, and one reported ecotourism, the cell was visualised with two quadrants representing agriculture, one representing game farming, and one representing ecotourism. This approach firmly shows the dominant land-use practices within each grid cell and may give insight into ecological behaviour of cheetah.

The resulting land-use map (Fig. 11A) indicates clear spatial differences between the two regions. Region 1 is predominantly characterised by livestock farming, followed by game farming, but with limited agriculture and ecotourism. In contrast, Region 2 is largely dominated by game farming, with smaller presence of the other land-use types. These differences in land-use are closely associated with underlying habitat characteristics. Region 1 primarily consists of grassland and semi-arid habitats, whereas Region 2 contains more thick vegetation, including shrubland and woodland systems [51][80]. Livestock farms are typically associated with more open landscapes, like Region 1, that support grazing species such as sheep and goats [37]. In contrast, areas with denser and less disturbed vegetation, Region 2, are more conducive to wildlife-based land-uses, such as game farming and ecotourism, where habitat structure supports higher biodiversity and a more natural ecosystem [61].

Differences in land-use practices are further reflected in fence types (Fig. 11B). Participants reported the use of various fencing types, including no fencing, small strand (2-4) fencing, game fencing, and predator-proof fencing, along with the proportion of each fence type. Using a similar spatial representation as for land use, comparisons between land-use and fencing types can be made. The livestock dominated areas more commonly use small-strand fencing, whereas game fencing is more prevalent in Region 2. While game fencing can support wildlife management and reduce certain types of human–wildlife conflict, it also contributes to landscape fragmentation. Impermeable fencing restricts the movement of wide-ranging species, including cheetah, limiting access to prey and habitat. At the same time, this fencing can confine predators within fenced areas, potentially increasing the likelihood of human–wildlife conflict.

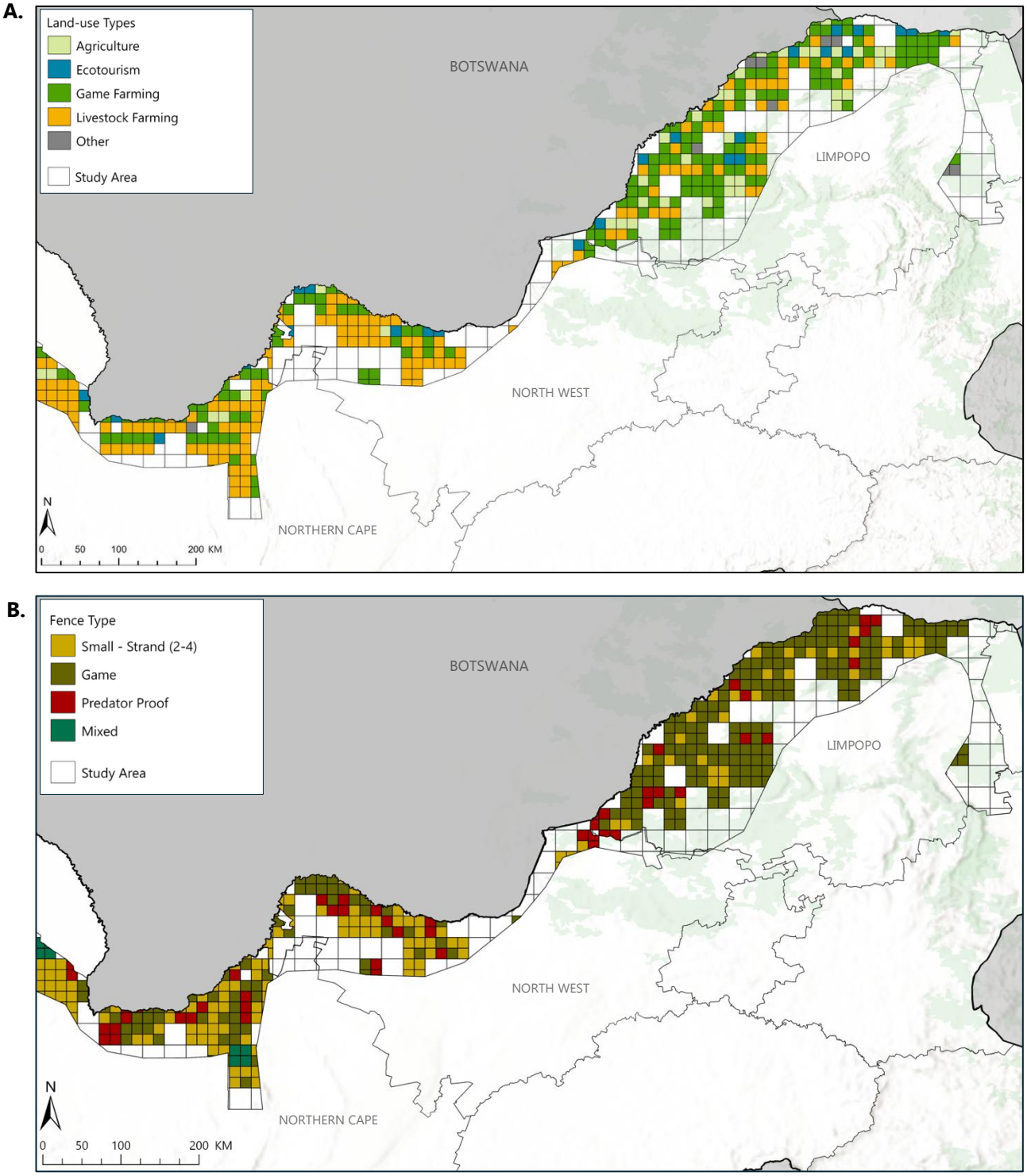

***Fig. 11**: The different land-use (**A**) and fencing (**B**) types mapped across the study area as stated by the farm occupants.*

4.2 Individuals

The following sections examine the presence, numbers, and movement patterns of cheetah populations within these fragmented landscapes. The **central objective** of the FRCC, establishing robust baseline numbers of free-roaming cheetah in South Africa, required accurate identification and individual recognition of cheetah. A total number of 5,212,796 images were uploaded in TrapTagger ($N_{Region1}$ = 2,823,736; $N_{Region2}$ = 2,389,060), an open-source AI-assisted web application designed for wildlife camera-trap data [99].

4.2.1 Dataset preparation

TrapTagger offers an initial workflow for processing large camera-trap datasets and facilitating species identification and individual-level differentiation. Within the software, the images collected from the field were uploaded according to QDGC (per year) into one project called a "survey". Each new survey was named according to "FRCC_year_province_grid" (e.g. FRCC_2023_LP_L3), resulting in a total of 198 surveys across 93 unique grid cells. To upload the 5.2 million images, approximately 2,700 hours (113 full, 24h days) was spent on ~12.5 TB of data and a total of 621 hours (26 full, 24h days) was spent on subsequent analysis by hand.

In preparation for image analysis, Static Detection was applied to filter out empty images. TrapTagger automatically draws sighting boxes around detected animals/objects that are believed to have triggered the camera. These boxes make Species Detection easier for the assigned annotator (i.e. the person manually labelling the images). During the Static Detection, TrapTagger would present the annotator with groups of images where the camera was triggered at the same space on the image multiple times. The annotator could then verify whether an animal triggered this space continuously (reject as Static Detection) or if it was an inanimate object such as a branch or grass (accept Static Detection).

After the Static Detection, the annotator supplies the survey with an annotation set. An annotation set is a default set that consists of the species most likely to occur in your study area. By adding this set, TrapTagger knows which species it should look for inside the images. Within the census the 'sub-Saharan Africa' species classifier set was used. This 'sub-Saharan Africa' set still missed various species marked as highly present or important, resulting in the addition of the hyrax, ground squirrel, vulture, kori bustard, owl, meerkat and dromedary. Besides adding species, the group of 'Vehicles, humans, livestock' was manually subdivided into 'human and vehicle', 'goat and sheep', 'horse and donkey', and 'cattle'.

Once the annotation set was applied, an 'area' needed to be established, which can be a self-determined area. Within TrapTagger every survey was placed in an area based on their placement in either Region 1 or Region 2 (Fig. 3)

4.2.2 Image analysis

By default, approximately 70% of the images have been annotated by AI and the remainder (30%) manually. However, to increase the accuracy of the data, around 70% was **manually** annotated instead. Analysis of the 5.2 million images started with Species Group Labelling. Rather than reviewing individual photos, TrapTagger groups images into clusters. Clusters are groups of images where each group is ≤30 minutes apart, treating long-lasting individual presence (e.g., pacing back and forth) as one event rather than separate detections [95]. A total of 266,092 clusters has been analysed in the Group Labelling phase, with cluster size differing from one image to just over 1,000 images (e.g. due to antelope or cattle herd). Group Labelling involves the classification of groups, such as antelope, rather than the specific antelope species. This Group Labelling is mandatory to be able to continue to the next steps of the analysis, while the specific Species Labelling is optional but regarded as highly important for the census. The groups that were de-grouped and classified as species specific were 'vehicle, human, livestock', 'small and medium cats' 'hyaena', 'jackal', 'antelope', 'bird', 'pig', 'primate', 'mongoose' and 'sundry'. Other, already species-specific clusters were also double checked manually for a more accurate species count. These consisted of lion (*Panthera leo*), leopard (*P. pardus*), cheetah (*A. jubatus jubatus*) and wild dog (*Lycaon pictus*).

Whenever a species was mentioned as present in a study area where the annotator found a low probability of that being true, these images were confirmed manually. If, during the Species Labelling phase, a camera was marked as knocked down, a knocked down analysis would follow immediately after. For the duration of the census, cameras were actively taking pictures (i.e. cameras not broken, batteries empty or knocked down) for 58,768 days, of which cameras were knocked down for 797 days (1%).

In Region 1, cameras were active for a total of 29,395 days across 165 sites, equalling to an activity of 178 days/site. Region 2 had cameras active for 29,373 days across 155 different sites, resulting in an average site active period of 190 days/site. After de-grouping and labelling within each group or species, an AI Check, Multi-species Differentiation, Sighting Box Correction and, if cheetah seen, Individual Identification were executed in that exact order. The AI check presented with all the clusters where the AI disagreed with the labels manually given to the images. Hence this analysis was used as a control for the work that was done during the Species Labelling phase.

When multiple species appeared within a cluster during the Species Labelling phase, TrapTagger draws a large sighting box around the entire image, requiring the annotator to scroll through the cluster images to reveal the image with multiple species. If multiple species were indeed present, these clusters will be re-annotated during the Multi-Species Differentiation phase. The Multi-Species Differentiation takes all clusters labelled as containing multiple different species and presents them to the annotator to manually give each species its appropriate label. This analysis type is of high importance as it gives a more accurate count of the species, making it especially valuable for the predator species (Fig. 12).

Fig. 12*: A cluster containing eight images where cattle and warthog were labelled as present after de-grouping the groups 'Vehicles, humans, livestock' and 'pig' that were given during the Species Labelling phase.* ***A****. All individuals are currently labelled as cattle.* ***B****. All species labelling have been corrected and boxes not containing an animal have been removed.*

For each survey with cheetah presence (N_{survey} = 59, N_{grid} = 28), Individual Identification was executed. The first step consists of ensuring that each sighting box that supposedly contains cheetah, indeed contained a cheetah. Similarly, extra sighting boxes had to be added to the image containing a cheetah not spotted by TrapTagger. These practices were executed during the Sighting Box Correction analysis, where all clusters containing cheetah were presented to the annotator for corrections (Fig. 13).

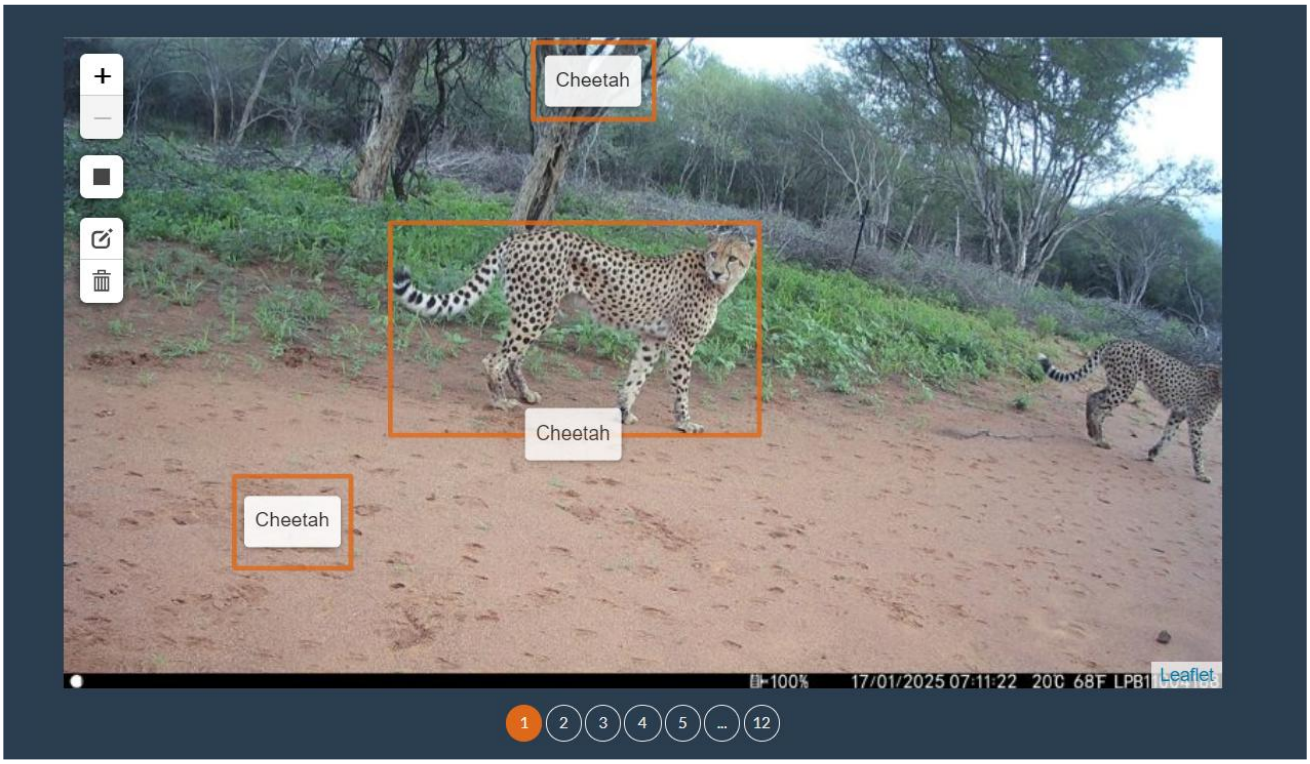

***Fig. 13**: Sighting Box Correction for a cluster with 12 images annotated to contain cheetah. Only one of the three boxes created contains a cheetah. The annotator needs to manually remove the two extra boxes, while adding a new box to the cheetah sighting for the individual on the right.*

4.2.3 Individual identification

The final step in data processing contained the actual Individual Identification of all clusters containing cheetah images ($N_{clusters}$ = 1,886; $N_{Region1}$ = 986; $N_{Region2}$ = 900). The identification process consists of two steps: Within Survey Identification and Between Survey Identification. The process starts with Within Survey Identification, where TrapTagger presents the same cluster next to each other to the annotator. By doing so, it was possible to check whether the cheetah seen on image one in the cluster, is the same as the individual seen on image two, etc (Fig. 14).

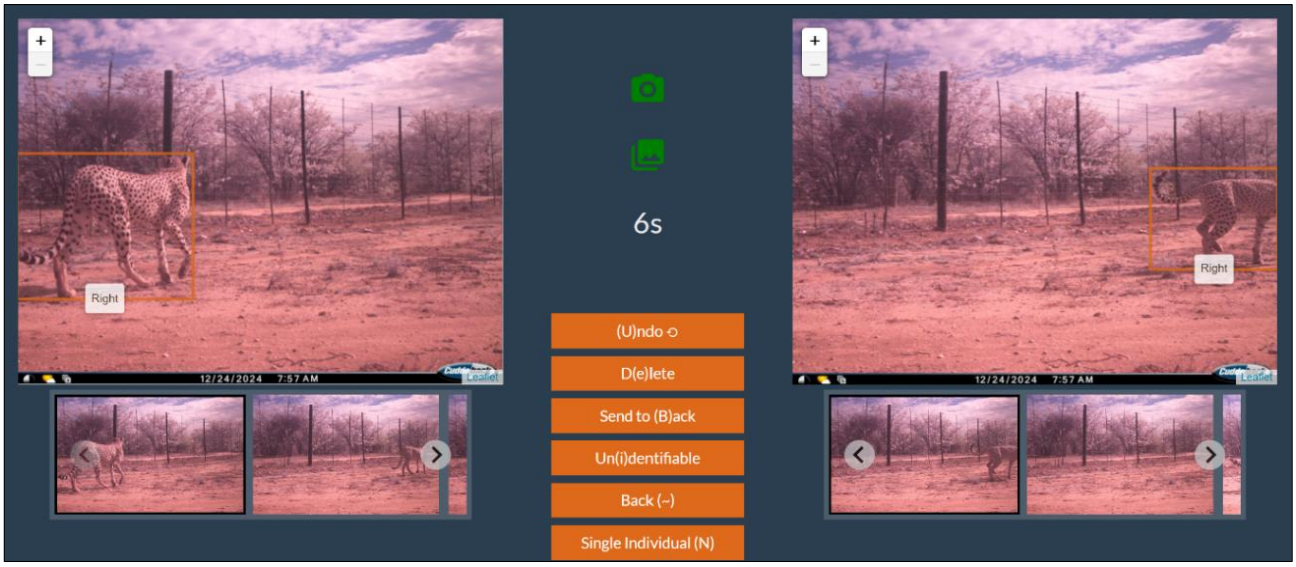

***Fig. 14**: The left image is photo number one, while the right image shows the second photo from the same cluster. They are taken six seconds apart by the same camera and both containing the right flank of a cheetah individual. The spot patterns are used to manually show that these are not the same individual, but a coalition of two brothers.*

The identification of cheetah was done with the help of their unique spot patterns. The focus for identification lies especially on the legs, shoulders and buttocks as these areas stretch the least after a heavy meal, during pregnancy or movement to maintain similar spot patterns (Fig. 15). This process was therefore done manually and was repeated for all clusters marked with cheetah present within a survey.

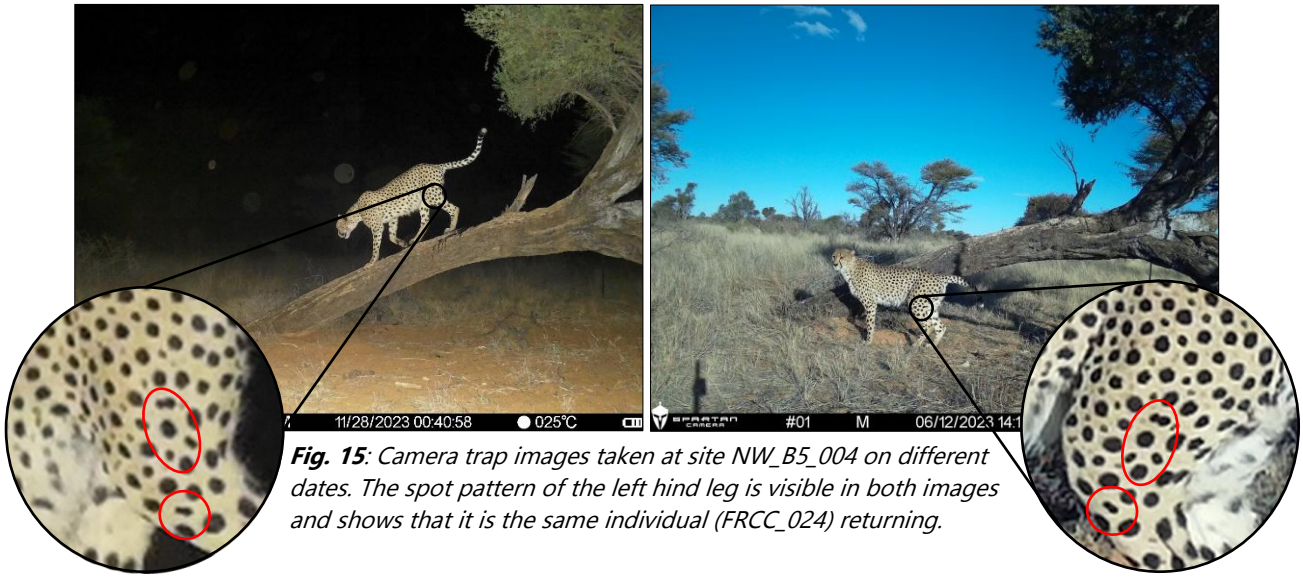

***Fig. 15**: Camera trap images taken at site NW_B5_004 on different dates. The spot pattern of the left hind leg is visible in both images and shows that it is the same individual (FRCC_024) returning.*

Once the Within Survey Identification finished, an additional analysis was executed: Between Survey Identification. With this identification analysis, all other surveys within the same area (i.e. Region 1 or Region 2) are compared to each other. This process shows all previously identified cheetah individuals within an area and compares them with the newly added individuals from the Within Survey Identification process. During this phase, built-in heatmaps for easy similarity detection were used to help decide if the individual is truly new, or if it was seen somewhere else within the area (Fig. 16).

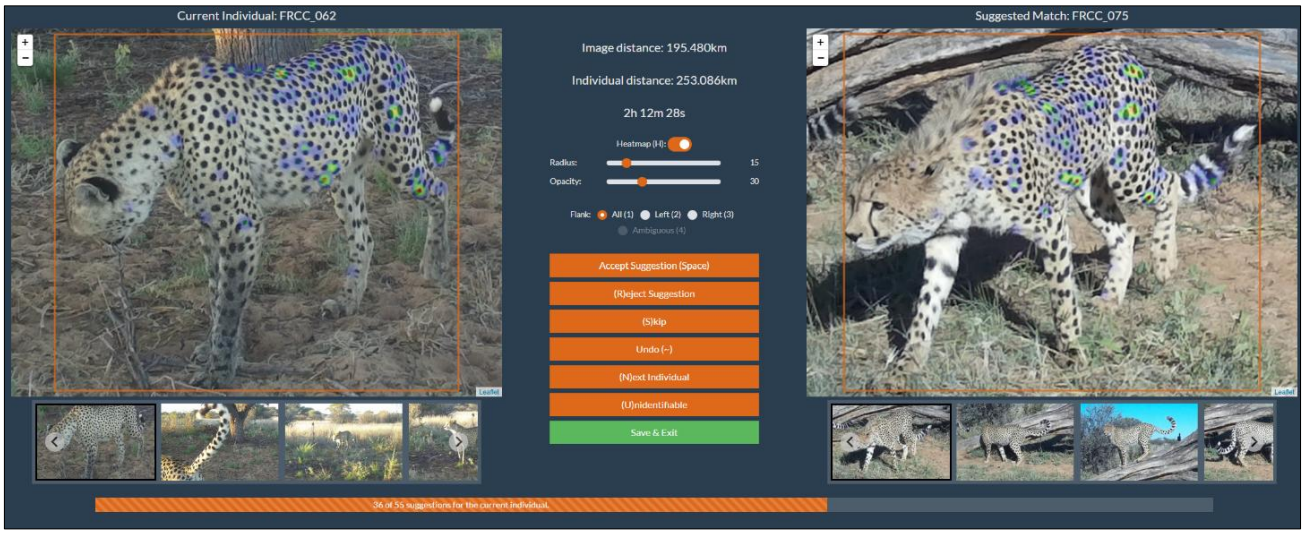

***Fig. 16**: The comparison between individual FRCC_062 (left) and FRCC_075 (right). The heatmap colouration shows the spot patterns that match the most. In this case little pattern overlap is seen and the highlighted spots do not match. It is not the same individual.*

4.2.4 Identified individuals

A total of 119 unique cheetah individuals (N_{camera} = 95, N_{collar} = 10, N_{report} = 14) were identified across the study area (Table 3), distributed between Region 1 (Northern Cape + North West; N = 58) and Region 2 (North West + Limpopo; N = 61). For standard population estimation, adults >24 months of age, of which 83 individuals were confirmed, were used for population counts. The confidence of every individual is rated on a scale of 1–5, with one indicating high identification uncertainty (e.g. few images, blurry images, only one flank, cubs) and five indicating strong confidence in the identification of that individual. This scaling is converted to percentages for each category in Table 3 with the formula:

$$Conf = \frac{Total\ confidence\ per\ category}{(Total\ individuals\ per\ category * 5)} * 100\%$$

For example, there are 30 adult males in Region 2, with a confidence sum of 131 (all confidence rates for each adult male in Region 2 added together). The maximum confidence of 30 males (if all individuals have a surety of 5) is 30*5. This would create the formula:

$$Conf = \frac{131}{(30 * 5)} * 100\% = 87\%$$

The total number of individual cheetah present in the study area were identified, how often they were recaptured at sites, what times they were active and their possible mortality rate. A cheetah ID kit (Appendix VI) was created which supplied individual flank images, estimated age and sex, location and sighting timeframe.

***Table 3**: The number of identified individuals across the study area subdivided into age (cub ≤ 12 months; sub-adult 12 < x < 24 months; adult ≥ 24 months) and sex categories. Confidence in identification is marked in %.*

Category	Region 1 (NC + NW)	Region 1 Confidence	Region 2 (NW + LP)	Region 2 Confidence	Total
Adult Males	20	98%	30	87%	50
Adult Females	11	91%	17	88%	28
Adult Unknown	2	50%	3	69%	5
Adult Subtotal	**33**	**93%**	**50**	**86%**	**83**
Sub-adult Males	3	100%	2	100%	5
Sub-adult Females	4	95%	2	100%	6
Sub-adult Unknown	2	40%	2	60%	4
Sub-adult Subtotal	**9**	**84%**	**6**	**87%**	**15**
Cub Unknown	**16**	**46%**	**5**	**20%**	**21**
Grand Total	**58**		**61**		**119**

4.2.5 Mortality

The census identified 58 individuals in Region 1 (N_{adults} = 33; $N_{sub\text{-}adults}$ = 9; N_{cubs} = 16), 10 of these individuals are confirmed dead, consisting of five adult males, one sub-adult female, one sub-adult of unknown sex, and three cubs. In Region 2, 61 individuals (N_{adults} = 50; $N_{sub\text{-}adults}$ = 6; N_{cubs} = 5) were identified, of which 14 are confirmed dead, consisting of 12 adults (seven males and five females), and two sub-adults of unknown sex. The confirmed individual mortality data consisted of a relatively small sample size (N = 17), with a pronounced sex

bias (N_{Female} = 5, N_{Male} = 12). Across both regions another four individuals were confirmed dead during the census period, but they could not be individually identified and thus not be linked to the ID kits.

For further insight into mortality causes, the mortality dataset was increased by adding confirmed deaths from 2021 and 2022. This resulted in an additional five individuals, making the total confirmed deaths 29 ($N_{Region1}$ = 14; $N_{Region2}$=15). The cause of death was confirmed in 15 out of 29 total mortalities. These deaths were mainly driven by human-related (anthropogenic) factors (Table 4; Fig. 17). A total of 13 individuals (86%) died from human-induced causes, including shooting, snaring, and vehicle collision, and two individuals died due to suspected predation. The cause of death remained unknown for 14 individuals.

***Table 4**: This table represents each confirmed death for all census identified individuals (FRCC_000) and individuals confirmed as deceased between 2021 and 2022 (n/a). Each row indicates the cause of death and its corresponding category. Individuals that were no longer recaptured along with their coalition or litter mates, were assumed dead.*

Individual	Age category	Sex	First seen	Last seen	Cause of death	Category of death
Region 1						
n/a	Adult	Male	2020/10/19	2021/07/16	Snare	Anthropogenic
n/a	Sub-adult	*n/a*	2021/02/16	2021/02/16	Roadkill	Anthropogenic
n/a	Adult	Female	2021/03/31	2022/02/04	Snare	Anthropogenic
n/a	Adult	Female	2021/08/03	2021/08/03	Unconfirmed	*n/a*
FRCC_025	Adult	Male	2023/08/01	2023/08/01	Disappeared	*n/a*
FRCC_026	Adult	Male	2023/08/01	2023/08/01	Disappeared	*n/a*
n/a	Sub-adult*****	*n/a*	2024/02/09	2024/02/09	Road fatality	Anthropogenic
FRCC_066	Adult	Male	2024/06/26	2024/09/03	Disappeared	*n/a*
FRCC_071	Cub	*n/a*	2024/07/04	2024/07/04	Disappeared	*n/a*
FRCC_073	Cub	*n/a*	2024/07/04	2024/07/04	Disappeared	*n/a*
FRCC_074	Cub	*n/a*	2024/07/04	2024/07/04	Disappeared	*n/a*
FRCC_084	Adult	Male	2024/07/19	2025/04/13	Disappeared	*n/a*
FRCC_085	Adult	Male	2024/07/19	2025/04/05	Disappeared	*n/a*
n/a	Sub-adult*****	Female	2025/08/16	2025/08/16	Road fatality	Anthropogenic
Region 2						
FRCC_001	Adult	Male	2021/05/16	2024/07/07	Shot	Anthropogenic
FRCC_008	Adult	Male	2021/07/29	2022/03/08	Disappeared	*n/a*
n/a	Adult	Male	2022/01/20	2022/01/20	Unconfirmed	*n/a*
FRCC_100	Adult	Female	2022/04/20	2024/07/22	Unconfirmed	*n/a*
FRCC_101	Adult	Female	2022/04/20	2024/09/17	Snare	Anthropogenic
FRCC_102	Adult	Female	2022/04/20	2023/07/22	Shot	Anthropogenic
FRCC_103	Adult	Male	2022/07/09	2022/08/17	Leopard	Predation
n/a	Sub-adult*****	*n/a*	2023/04/05	2023/04/05	Lion******	Predation
n/a	Sub-adult*****	*n/a*	2023/04/05	2023/04/05	Likely shot******	Anthropogenic
FRCC_038	Adult	Female	2023/11/19	2023/12/11	Likely shot	Anthropogenic
FRCC_044	Adult	Female	2023/12/07	2025/06/04	Unconfirmed	*n/a*
FRCC_114	Adult	Male	2024/03/26	2024/03/26	Disappeared	*n/a*
FRCC_049	Adult	Male	2024/05/01	2025/06/13	Road fatality	Anthropogenic
FRCC_050	Adult	Male	2024/05/01	2025/06/13	Road fatality	Anthropogenic
FRCC_052	Adult	Male	2024/05/01	2025/06/13	Road fatality	Anthropogenic

***** These individuals have been confirmed dead, but due to their found physical state, no proper identification could be done. This means that it is not known if these individuals are part of the ID kit created during the census

****** These individuals broke into a fenced game reserve where one was killed by lions, and the other on community lands.

Mortality rates are generally identified based on adults only. When looking at solely the adult mortalities observed within the census years (FRCC_000; 2022–2025, N = 17), a mortality rate of 15% in Region 1 and 24% in Region 2 was calculated, with 20% adult mortality throughout the study area. This means that one fifth of the known free-roaming population died during the 3.5-year census period. This figure reflects only the confirmed mortalities, so the true mortality rate is likely higher.

Estimated age at last detection was determined by combining averaged age observed at first sighting with the elapsed time between the first and last detection. In instances where individuals were first captured as cubs or sub-adults, the body size could be used as a marker for the estimated date of birth. These estimates were used directly to assign an approximate age in months. In cases where precise age estimations were not possible, individuals were assigned age values based on categorical age classes. Midpoint values were used for each class

(cub: 6 months; sub-adult: 18 months; adult: 39 months). For adult individuals, the midpoint value was based on a 30 – 48 month range. Individuals that are confirmed dead, had the time between first and last detection added to the determined age at first observation to obtain an estimated age at death.

As age estimates were partly derived from categorical classes (cub, sub-adult, adult), these values should be interpreted as approximations rather than exact ages. However, the use of midpoints and field-informed estimates provides a consistent and biologically realistic framework for comparative analysis. Average age at death was calculated using only confirmed mortalities and estimated ages at the time of death were summarised across individuals to obtain a mean value.

Within the managed metapopulation, the average lifespan of cheetah is 5.94 years (±2.81 years), with anthropogenic causes accounting for 17% of the ~500 data points recorded in these reserve populations, while non-anthropogenic causes accounted for 46% [82]. In contrast, the free-roaming individuals identified during the census exhibited a markedly earlier average age at death of 3.72 years. A study in Namibia strengthens these outcomes with their findings on Namibian farmlands where free-roaming cheetah have an ~84% risk of dying between independence and six years of age [53].

This reduced lifespan occurs despite the absence of major natural predators, such as lions (*Panthera leo*), within the free-roaming landscapes., suggesting that other factors, of which most likely anthropogenic pressures, play a significant role in limiting survival. Consequently, one fifth of the known South African free roaming population died during the 3.5-year census period. This figure reflects only the confirmed mortalities (20%), so the true mortality rate is likely higher.

Fig. 17*: Three individuals found dead. One after a suspected leopard attack (FRCC_103; left), one caught in a gin trap (n/a; both middle images) and one sub-adult road fatality found next to a busy gravel road (n/a; right).*

4.3 Telemetry

Data from 20 collared individuals ($N_{Region1}$ = 7; $N_{Region2}$ = 13; Table 5; Fig. 18) were used to gain insight into how individuals utilise the landscape beyond static detection records. As collaring of individuals was done opportunistically and undertaken by COT in response to human-wildlife conflict incidents, the number of collaring events in Region 1 during the field work period (2022 – 2025) were limited relative to Region 2. Therefore, six individuals with telemetry data from earlier deployments (2020 – 2021) by COT were incorporated for Region 1 to ensure an adequate sample size. Using older satellite data from previously collared cheetah was deemed appropriate, as the interval between collaring and the start of the census was shorter than a free-roaming cheetah's typical lifespan (avg. 4.6 years, [97]). Therefore, these records likely reflect comparable movement based on land-use conditions to those during the study period.

***Table 5**: All collared individuals between 2020 – 2025 including the monitoring period during which the individual was fitted with the collar. The collars with outcome 'ongoing' are set to the census' end date (31-12-2025) as that is the last date used for the analysis, but the collar is still active.*

Individual	Collar	Sex	Start Date	End date	Active (days)	Mean Daily Distance (km)	Spatial extent (km^2)	Outcome
Region 1								
n/a	AWT 4213	Male	2020/10/19	2021/07/16	270	4.34	618	Mortality
n/a	AWT 4154	Male	2021/03/13	2022/06/14	458	5.39	6,171	Drop-off
n/a	Tel 723586A / AWT_4709	Female	2021/03/31	2022/02/04	310	1.96	173	Mortality
n/a	Tel 723419A	Male	2021/06/02	2021/10/13	133	6.89	2,490	Drop-off
n/a	Tel 723587A	Male	2021/05/25	2021/12/04	193	6.37	5,126	Collar fail
n/a	Tel 721907A	Female	2021/08/04	2022/06/20	320	6.04	4,991	Drop-off
FRCC_039	AWT 4709	Female	2022/03/26	2023/06/21	452	3.73	1,945	Drop-off
Region 2								
n/a	AWT 4679	Male	2022/01/20	2023/01/26	371	5.22	2,971	Drop-off
FRCC_100	AWT 5682 / 5681	Female	2022/04/24	2024/07/22	819	2.89	1,292	Mortality
FRCC_103	AWT 5681	Male	2022/07/09	2022/08/17	40	1.55	1.4	Mortality
FRCC_101	AWT 5680	Female	2022/08/04	2024/09/17	775	4.41	1,589	Mortality
FRCC_102	AWT 5683	Female	2022/08/04	2023/07/22	352	3.72	1,286	Mortality
FRCC_038	ST 1172	Female	2023/11/22	2023/12/02	10	4.61	33.8	Collar fail
FRCC_001	ST 1233	Male	2024/02/21	2024/07/07	136	6.21	213	Mortality
FRCC_044	ST 1269	Female	2024/06/20	2025/06/04	348	3.76	748	Mortality
FRCC_050	AWT 7898 / 10056	Male	2024/08/27	2025/06/23	300	7.16	559	Mortality
FRCC_064	AWT 10112	Male	2025/06/11	2025/12/31	203	6.80	1,117	Ongoing
FRCC_096	AWT 10342	Male	2025/09/11	2025/12/31	111	4.10	169	Ongoing
FRCC_097	AWT 10339	Male	2025/08/31	2025/10/08	38	5.28	32	Collar fail
FRCC_095	AWT 10340	Male	2025/10/13	2025/12/31	78	6.73	293	Ongoing

Along with limited sample sizes, tracking duration was often constrained by mortality or collar failure. Traditional approaches for estimating home range, such as kernel density estimation (KDE), rely on assumptions of smooth and continuous space use (i.e. no barriers) and require relatively large, temporally extensive datasets to produce reliable estimates [31]. Similarly, Minimum Convex Polygons (MCP) may overestimate space use due to inclusion of outlier movements [12][83]. Given these limitations, estimates are more appropriately interpreted as spatial extent rather than true home range, reflecting the geographical area utilised during the monitoring period without assuming stable, long-term use.

Accordingly, spatial extent was estimated using a cluster-based convex hull approach, in which location data were partitioned using k-means clustering (which groups data points into clusters based on how close they are to each other) and convex hulls were generated for each cluster before being merged to represent overall spatial extent. Similar multi-hull approaches have been shown to better reflect patterns of space use by constructing polygons around localised groups of points and reducing the inclusion of unused areas [36][15]. Importantly, this approach allows for standardised comparisons across individuals and regions, while minimising methodological bias introduced by uneven data quality.

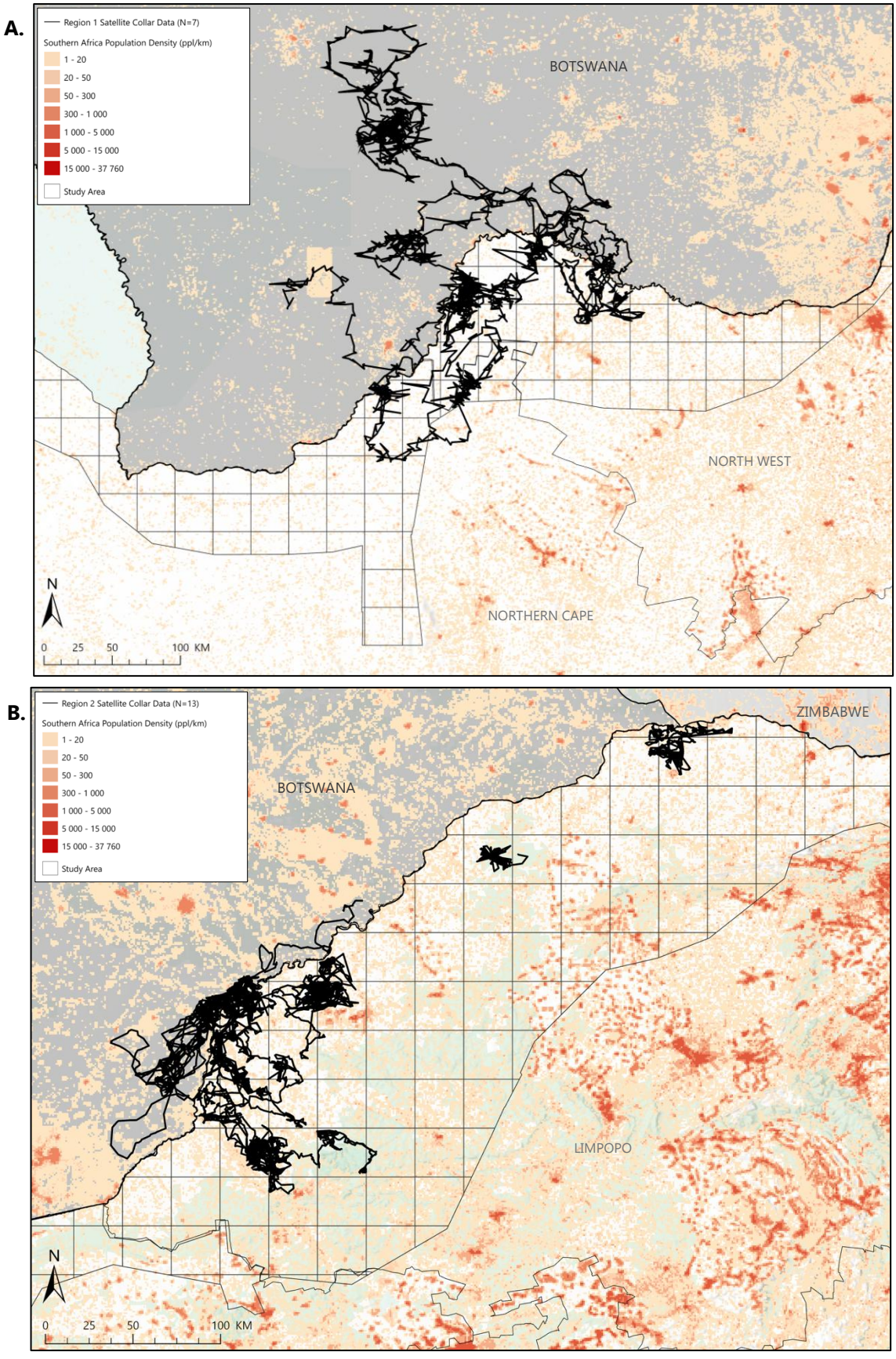

***Fig. 18**: All collared individuals per region. Region 1 (**A**) shows a larger movement range with more opportunities to move into neighbouring countries. Region 2 (**B**) shows how human population causes restricted movement, with individuals crossing the country border eventually being blocked from further inland movement.*

To account for differences in monitoring duration and sampling effort, spatial extent estimates were interpreted alongside tracking duration (days) and movement metrics, specifically mean daily distance travelled. Daily movement distances were calculated by summarising step lengths (i.e. the distance between consecutive GPS fixes per individual per day) and provided an additional measure of space use. This allowed a more nuanced interpretation of spatial extent, particularly where tracking periods are short, and helped distinguish between large extents resulting from extensive daily movement versus those with prolonged monitoring periods.

4.3.1 Distances travelled by individuals

Daily distance travelled was fairly similar between regions ($N_{Region1}$ = 4.78 km, $N_{Region2}$ = 4.46 km) with a nearly identical median value of ~3.6 km/day per individual (Fig. 19). This indicates, that on average, individuals in both regions exhibit comparable day to day movement in terms of travel distance. However, **similar values of distance do not necessarily imply equal range use**, as the total area an animal uses over time can differ.

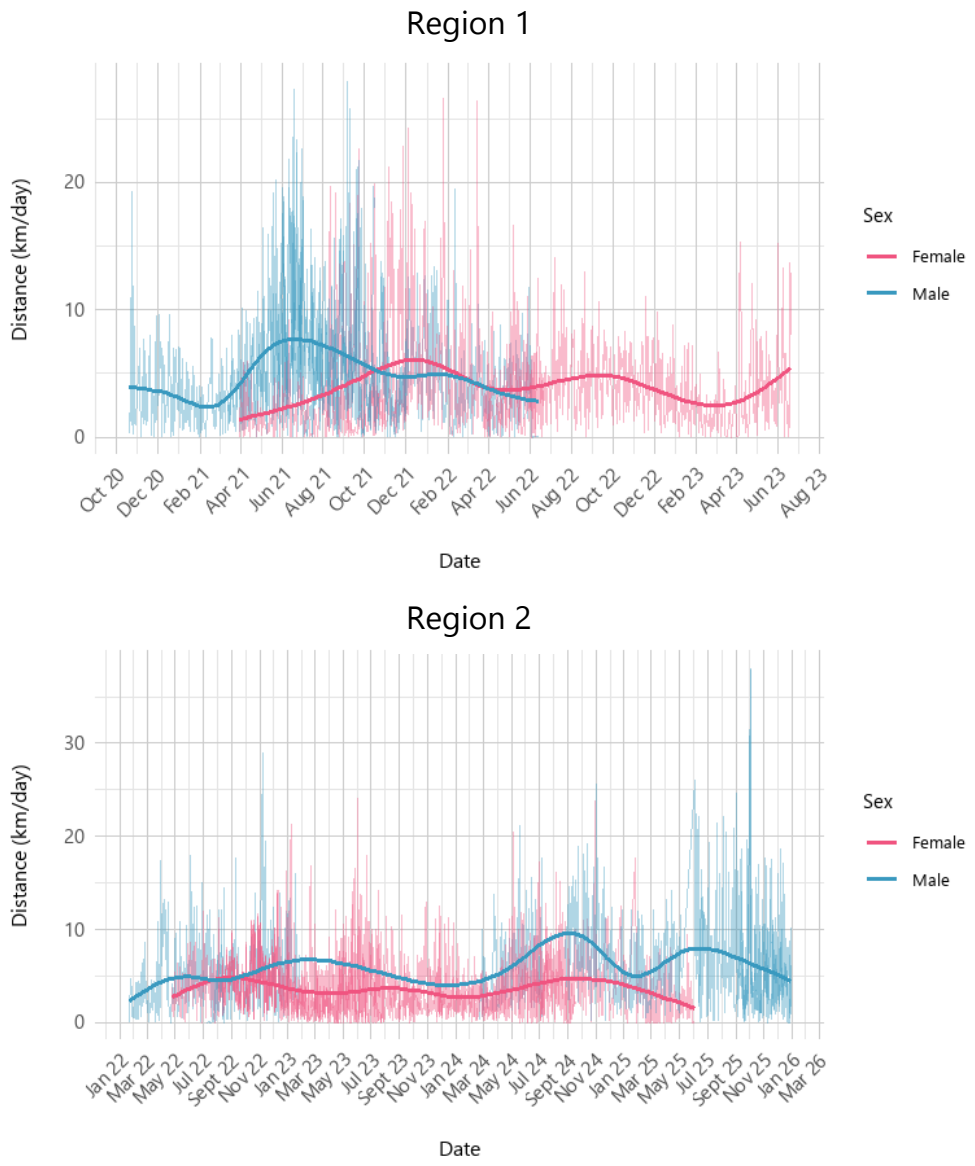

***Fig. 19**: Daily movement distances (km/day) of collared individuals over both regions. Thin lines show daily data for females (pink) and males (blue) accompanied by their respective trendlines. Both regions had a near identical median value of ~3.6km/day per individual.*

Despite the similarity in distance travelled, differences were found between the regions in movement variability when assessed at the individual level. To account for unequal sample sizes, individuals in Region 2 were randomly subsampled to match the number of individuals in Region 1. While typical levels of variability were similar between regions, the spread of variability (measured using the interquartile range (IQR), which represents the spread of the middle 50% of values) was much greater in Region 2 (IQR = 0.218) compared to Region 1 (IQR = 0.061). This indicates that movement behaviour in Region 2 is less consistent, with some individuals showing very stable movement patterns, while others displayed more variable behaviour that likely reflects differences in landscape structure (e.g. fragmentation and the presence of boundaries) that influence how individuals move through the environment.

In both regions, males and females exhibited occasional spikes in daily movement distances, with some movements exceeding 20 km/day. Sex-based differences in movement were present but not pronounced. Males tend to exhibit slightly higher variability and more frequent long-distance movements, while females showed more consistent daily movement patterns. However, the overall overlap between sexes suggests broadly similar movement behaviour.

It is important to note that monitoring duration differed substantially between the regions, with Region 2 contributing a larger number of tracking days ($N_{Region1}$ = 2,077 days, $N_{Region2}$ = 3,507 days). This increased sampling effort may influence the likelihood of capturing rare long-distance movements and should be considered when comparing variability between regions. Overall, these findings indicate that, no matter the region or sex, daily distance is relatively similar, meaning that differences in spatial extent between regions are unlikely to be driven by daily movement alone. Instead, landscape barriers and spatial constraints are likely to play a more significant role in shaping overall space use.

4.3.2 Spatial Extent

Spatial extent showed how far individuals moved from their release point during the monitoring period. In Region 1, spatial extent varied considerably among individuals (N = 7), ranging from relatively small areas (~100 km^2) to extremely large extents exceeding 6,000 km^2 (Fig. 20). These extreme values were facilitated by cross border movement into the Kgalagadi District of Botswana (Fig. 18A), with larger, less fragmented farmland areas that allow for more extensive movement before encountering human settlement barriers [94].

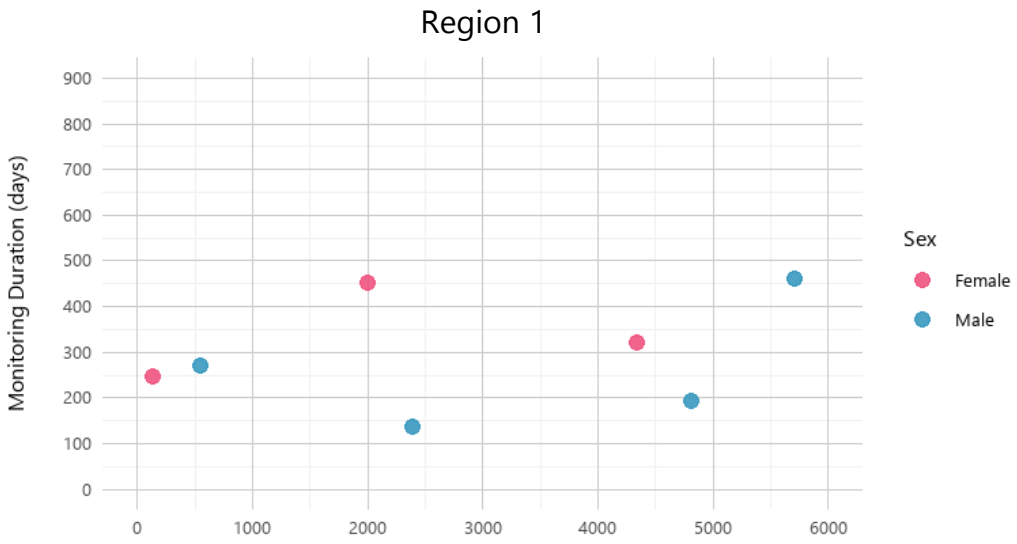

***Fig. 20**: Region 1 spatial extent and monitoring duration plotted by sex indicating the relatively mixed estimates.*

Such large extents likely reflect atypical dispersal or exploratory behaviour rather than stable space use, as similar patterns were observed in the above individuals coalition mate (~5,000 km^2) and an unrelated female (~4,000 km^2). However, these observations remain ecologically significant, as they suggest that Region 1 may represent one of the few landscapes within the free-roamer range where large-scale, unrestricted movements of this magnitude is still possible. This highlights the role of landscape permeability in facilitating connectivity and long-distance movement, even if such behaviour is not representative of typical space use [42][57].

There is no clear relationship between monitoring duration and spatial extent, as individuals tracked for shorter periods often exhibited extents comparable to, or larger than those monitored for longer durations. This is consistent with the daily movement patterns observed, where individuals displayed frequent long-distance movements. As a result, spatial extent in Region 1 appear to reflect broad and flexible space use rather than constrained movement in clearly enclosed areas.

In contrast, Region 2 exhibits generally smaller and more constrained spatial extents, with most individuals (N = 13) occupying areas below 1,500 km^2 (Fig. 21). The few individuals who occupied ranges exceeding this threshold moved beyond the region into Botswana, particularly into sparsely populated areas running east along the border. Further movement into Botswana is still largely restricted by high human settlement density, particularly in the South-East district (Fig. 18B). However, movement along the South Africa-Botswana border towards PAs such as Gonarezhou (Zimbabwe) and Kruger TP (South Africa, Zimbabwe, Mozambique), seems likely and appear to be one of the few possible movement corridors keeping the Region 2 population connected within South Africa.

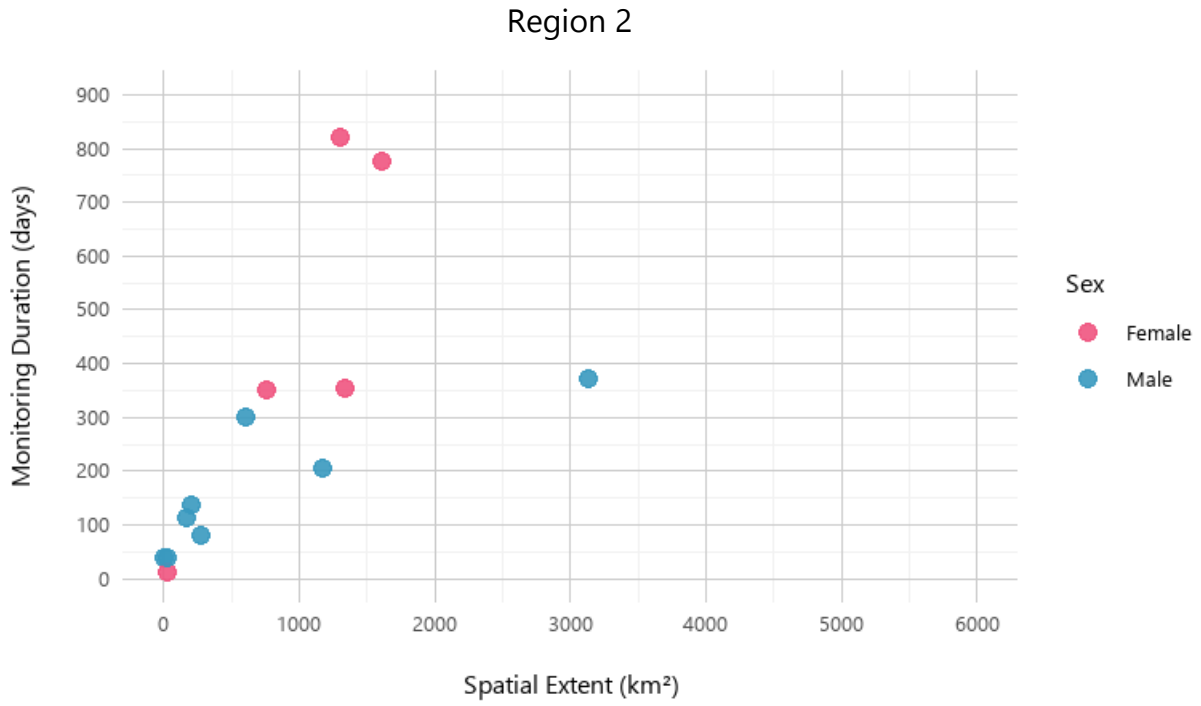

***Fig. 21**: Region 2 spatial extent and monitoring duration plotted by sex indicating the relatively mixed estimates.*

Although some individuals with longer monitoring durations showed moderately larger extents, the overall spread remained limited compared to Region 1. The predominance of game fencing in Region 2 likely imposes structural constraints on movement, restricting the extent to which individuals can range. Consequently, spatial extent in this region is more confined and consistent across individuals.

Variation in spatial extent among females may also reflect reproductive behaviour during the monitoring period. Female cheetah with dependent cubs typically reduce their movement while denning and raising young. As a

result, spatial extent estimates derived during these periods are likely to be smaller and may not represent the full extent of space use under non-reproductive conditions. This is consistent with the observed movement data, where females generally exhibited more constrained daily movement compared to males. Periods of reduced movement associated with denning would therefore contribute directly to smaller spatial extents.

The relationship between spatial extent and monitoring duration (Fig. 22) further highlights the importance of extended tracking periods for reliable estimates. Individuals with shorter monitoring periods (often due to mortality or collar failure) tend to show highly variable spatial extents, which may underestimate or inconsistently represent true space use. In contrast longer monitoring durations appear to produce more stable and representative estimates.

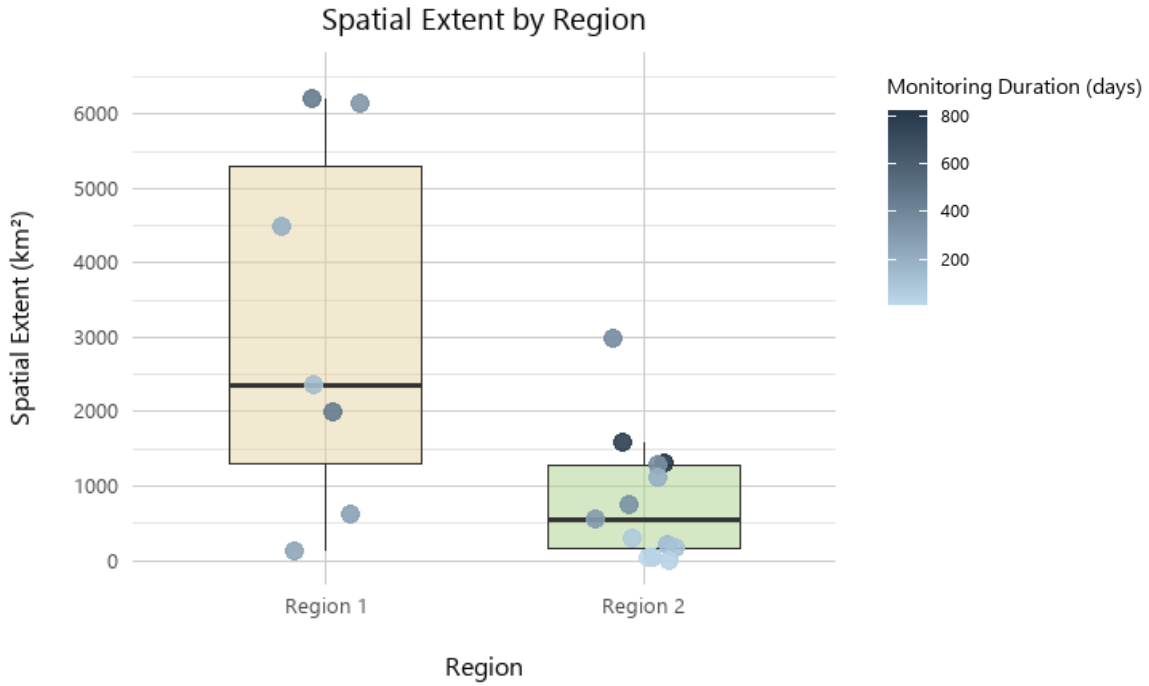

***Fig. 22**: Spatial extent by region concluded from a cluster based convex hull approach. Points represent individuals and are shaded by monitoring duration (days). Region 1 shows larger and more variable space use, while Region 2 shows low variability despite differences in monitoring duration.*

Given these limitations, subsequent analyses were restricted to a subset of individuals ($N_{Region1}$ = 5, $N_{Region2}$ = 7) with monitoring durations exceeding 200 days to derive representative estimates of spatial extent for each region. **The median spatial extent in Region 1 was approximately 2,032 km², compared to 1,332 km² in Region 2.** These findings suggest that human density, land-use practices and associated fence types play a key role in the shaping of cheetah movement and range establishment. Open landscapes and permeable livestock-dominated systems support wider-ranging movement, while more managed, fenced wildlife systems constrain movement.

4.4 Dietary Results

4.4.1 Host/Prey hair cross-section analysis

During the initial phase of the census, hair cross-section analysis was considered an adequate tool for determining host and prey species [49]. Unfortunately, as the analysis progressed, confidence in the results dropped. Hair cross-section identification can wrongly identify species with similar hair structure, completely miss rare species and vary due to observer bias [87][24]. Of the 164 samples collected, 73 underwent hair cross-section analysis.

4.4.2 Host hair cross-section and DNA sequencing comparison

To compare methods, 62 faecal samples of the original 73 samples that were washed for hair analysis and had adequate remaining material to perform DNA sequencing were used. Of these 62 samples, 15 showed no genetic material available. Thus, only 47 samples that have undergone both analyses and resulted in a host species finding, were compared (Table 6).

***Table 6**: Direct comparison of 47 samples that underwent both hair cross-section analysis and DNA sequencing for host species determination.*

Host Predator Species	DNA Analysis	Hair Analysis	Hair sample – false positive	Hair sample – false negative
Cheetah (*A.jubatus jubatus*)	34	39	23% (9)	-
Leopard (*P. pardus*)	3	4	75% (3)	-
Brown Hyaena (*Parahyaena brunnea*)	2	0	-	100% (2)
Caracal (*Caracal caracal*)	1	0	-	100% (1)
Black-backed jackal (*C. mesomelas*)	7	0	-	100% (7)
Chacma baboon (*Papio ursinus*)	0	3	100% (0)	-
Nothing found	0	1	100% (0)	
	47	**47**		

Along with the host species comparison, prey species results also differed between the analyses, with only 23% of 38 comparable samples agreeing on the result. It is worth noting that some DNA could have been lost during the washing process, which must be considered when looking at the comparison between analyses. As a result of the above comparison, hair cross section analysis was discarded in favour of DNA sequencing that offers results with definitive identification at the molecular level.

4.4.3 Prey DNA sequencing results

DNA sequencing was done on all 164 faecal samples ($N_{Region1}$ = 138; $N_{Region2}$ = 26) at the Jonah Ventures lab. Of these, 106 samples have confirmed cheetah as the host species (65%) of which prey DNA was detected in 54 samples ($N_{Region1}$ = 46; $N_{Region2}$ = 8). Within one scat sample, multiple prey species can be identified. All identified prey belonged to game species, which were categorised into either small (<100 kgs) or large game (≥100 kgs; [75]) as shown in Table 7. This categorisation was used to check for small versus large game preferences and directly compare with camera trap dominant game species captured around the scat collection area.

Spatial buffers were generated around all confirmed cheetah scat collection points (Fig. 23) to represent the potential span of daily movement across different land-use types (section 4.3). Because the exact time of defecation was unknown, buffers were designed to represent the area within which an individual could

reasonably have moved and eaten prior to sample collection. Although some of these areas included cattle, goat or sheep farms, **none of the scat sampled contains livestock DNA**.

***Table 7**: All game species found in cheetah faecal samples based on DNA sequencing categorised into small and large species. The nr. samples indicates how often that species has been identified in cheetah scat. One faecal sample can have multiple prey detected in it*

Game Species	Avg. weight (kg)	Category	Nr. samples
Blesbok (*Damaliscus pygargus phillipsi*)	80	Small	1
Blue wildebeest (*Connochaetes taurinus*)	260	Large	5
Bontebok (*Damaliscus pygargus pygargus*)	60	Small	8
Burchell's zebra (*Equus quagga burchellii*)	290	Large	1
Cape porcupine (*Hystrix africaeaustralis*)	17	Small	2
Duiker (*Sylvicapra grimmia*)	20	Small	4
Eland (*Taurotragus oryx*)	550	Large	6
Impala (*Aepyceros melampus*)	50	Small	6
Kudu (*Tragelaphus strepsiceros*)	240	Large	9
Oryx (*Oryx gazella*)	200	Large	1
Red hartebeest (*Alcelaphus buselaphus caama*)	130	Large	8
Scrub hare (*Lepus saxatilis*)	3	Small	2
Springbuck (*Antidorcas marsupialis*)	40	Small	8
Steenbok (*Raphicerus campestris*)	12	Small	1
Waterbuck (*Kobus ellipsiprymnus*)	220	Large	1

To reflect the ecological movement differences between regions, these base buffer sizes were calculated using a movement-based approach, defined as the sum of the median and upper quartile (75th percentile) of daily movement distances for each region ($N_{Region1}$ = 9.46 km, $N_{Region2}$ = 9.33 km). The base buffers in Region 1 were increased by 20% to account for generally greater spatial extent, while buffers in Region 2 were reduced by 20% to reflect more spatially restricted movement associated with a more fragmented landscape. This modest proportional adjustment is supported by the presence of long-distance movements observed in Region 1, compared to more constrained and consistent movement patterns in Region 2 (section 4.3.2). This resulted in final buffer distances of ~11.4 km in Region 1 and ~7.4 km in Region 2. Individual buffers were subsequently dissolved within each region to produce a continuous spatial extent representing the cumulative area of potential cheetah movement.

Of the 54 samples with game DNA present, 29 (54%) matched the dominant game type that was observed on camera traps, meaning that cheetah preyed on what was most abundant locally, regardless of whether the scat deposited was on a livestock farm. In the 25 samples where cheetah ate prey not of the local dominant type, large game species were featured as mostly eaten, even when small game was more common in the area. However, this does not necessarily prove a strong preference for large game. DNA sequencing cannot distinguish adults from juveniles, so many 'large game' detections may represent young animals, effectively aligning them with small game in size and vulnerability. **At least 52% of the samples with prey detected in the scat were small game species**.

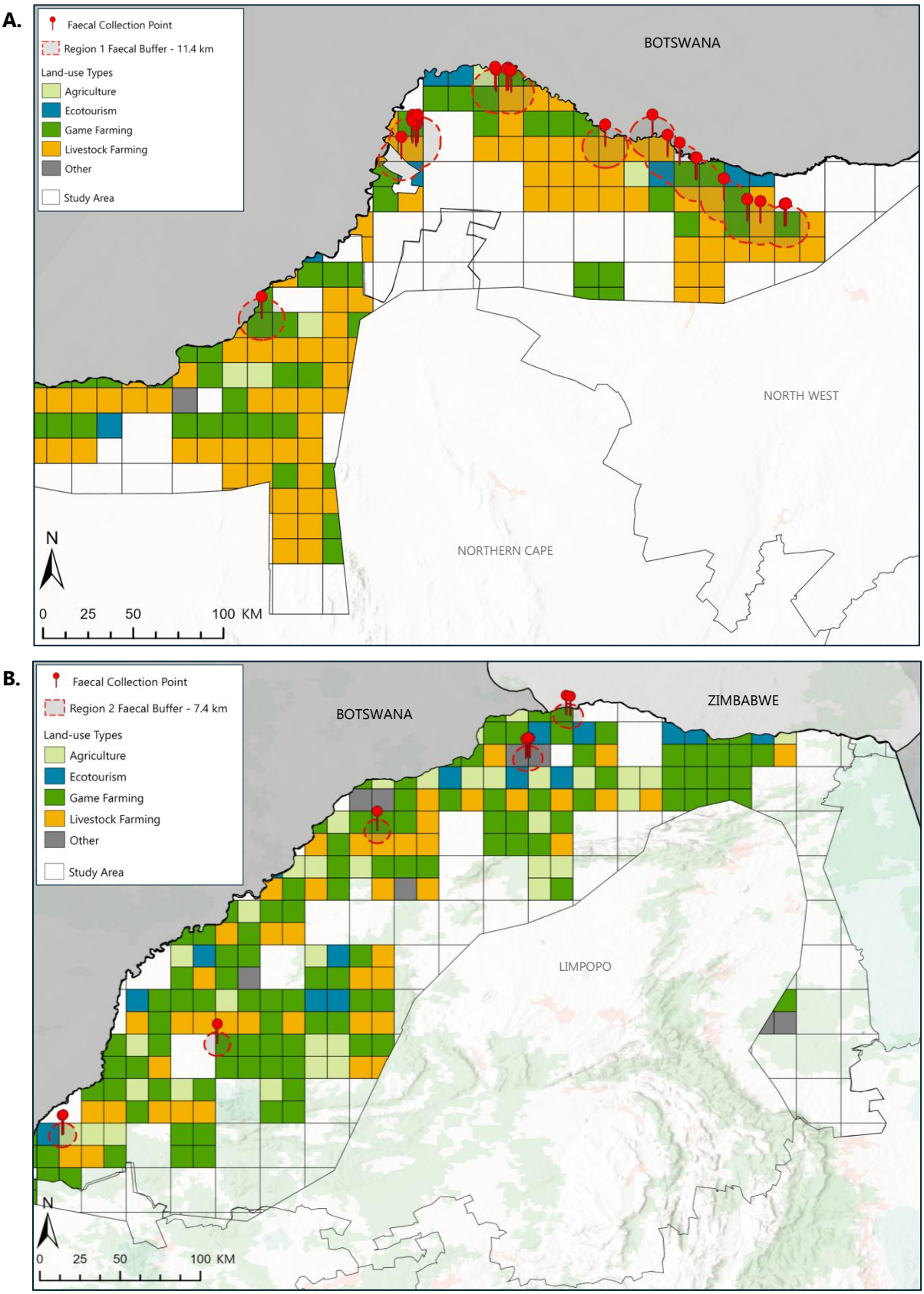

***Fig. 23**: Confirmed cheetah scat collection locations (black) as an overlay of different land-use types in Region 1 (A) and Region 2 (B). The buffer around the collection point provides an indication of the lands on which the cheetah might have hunted before defecating.*

4.5 Activity behaviour

4.5.1 Revisitation rate

The revisitation rate measures how frequently a cheetah individual returns to the same sentinel site. The rate is calculated as the average days between recaptures on camera across clusters for individuals or coalitions that were detected more than once at a site. Knowing the revisitation rate of cheetah captured on camera gives an indication of their movement behaviour and site use. These outcomes are essential for the census to estimate movement range, identify key habitat areas and possible conflict hotspots.

The revisitation rate analysis was focused solely on the adult individuals seen on camera traps (N = 66) since sub-adults and cubs that are still with their mother are dependent on her movement. Individuals that are a part of a coalition or a female with a litter are seen as one group as they revisit sites together. After removing individuals that have not been recaptured at any given site, 18 groups in Region 1 (10 single individuals, five coalitions and three litters including the mother) across 24 different sites and 16 groups in Region 2 (12 single individuals and four coalitions) across only 15 sites were left (Fig. 24).

In Region 1, analysis of these 18 groups resulted in an average recapture rate of 164 days between visits, ranging from 2-440 days, with multiple individuals taking on average more than 400 days between revisits (Fig. 24A). Region 2 exhibited more frequent recaptures, averaging 128 days (ranging between 3–378 days), with only one occurrence above 300 average days between clusters (Fig. 24B). Notably, no individuals from either region were recaptured in the alternate region, indicating no evidence of cross-regional movement during the study period. These patterns suggest greater dispersive behaviour in Region 1 compared to Region 2, supported by the representative spatial extent of 2,032 km^2 and 1,332 km^2 respectively (section 4.3.2).

The Region 1 individuals collared during the FRCC were never captured on camera, however, in Region 2, four of the 13 collared individuals were captured on camera trap during the census, all of whom have since died. Seven of the total collared individuals in Region 2 have confirmed mortalities, two are unconfirmed and four are alive. This high mortality of the collared cheetah makes the data for direct comparison of a collared individual with its camera trap recaptures insufficient. However, by plainly observing the movement pattern of all collared individuals, constrained space use can be observed for Region 2 (section 4.3), supporting the camera trap recapture analysis: Region 2 individuals have more restricted movement compared to Region 1 individuals.

The reason for these regional differences in cheetah revisitation rates likely stems from a combination of ecological and anthropogenic factors based on habitat preference and landscape barriers. Human population density plays a key role as fig. 3 reveals that the cheetah IUCN range in Region 2 is encircled by high human density areas, making it an 'island population'. This effectively isolates the Region 2 population, with no dispersal to Region 1 but limited movement only into bordering countries (e.g. Botswana or Zimbabwe). Additionally, Region 1's landscape (desert and shrubland) is more appropriate for livestock farming, while the farms in Region 2 mostly consist of game farms, with denser vegetation [37][61] (Fig. 11A), attracting cheetah and other large predators (≥ 15kg) due to a higher abundance of preferred prey species (Table 8). However, game farms generally use large game-proof fencing to keep their stock inside versus small strand fencing often used for livestock. These game fences are often impermeable, only making passage possible due to holes in or under the fencing, increasing fragmentation and restricting movement in Region 2 (Fig. 11B).

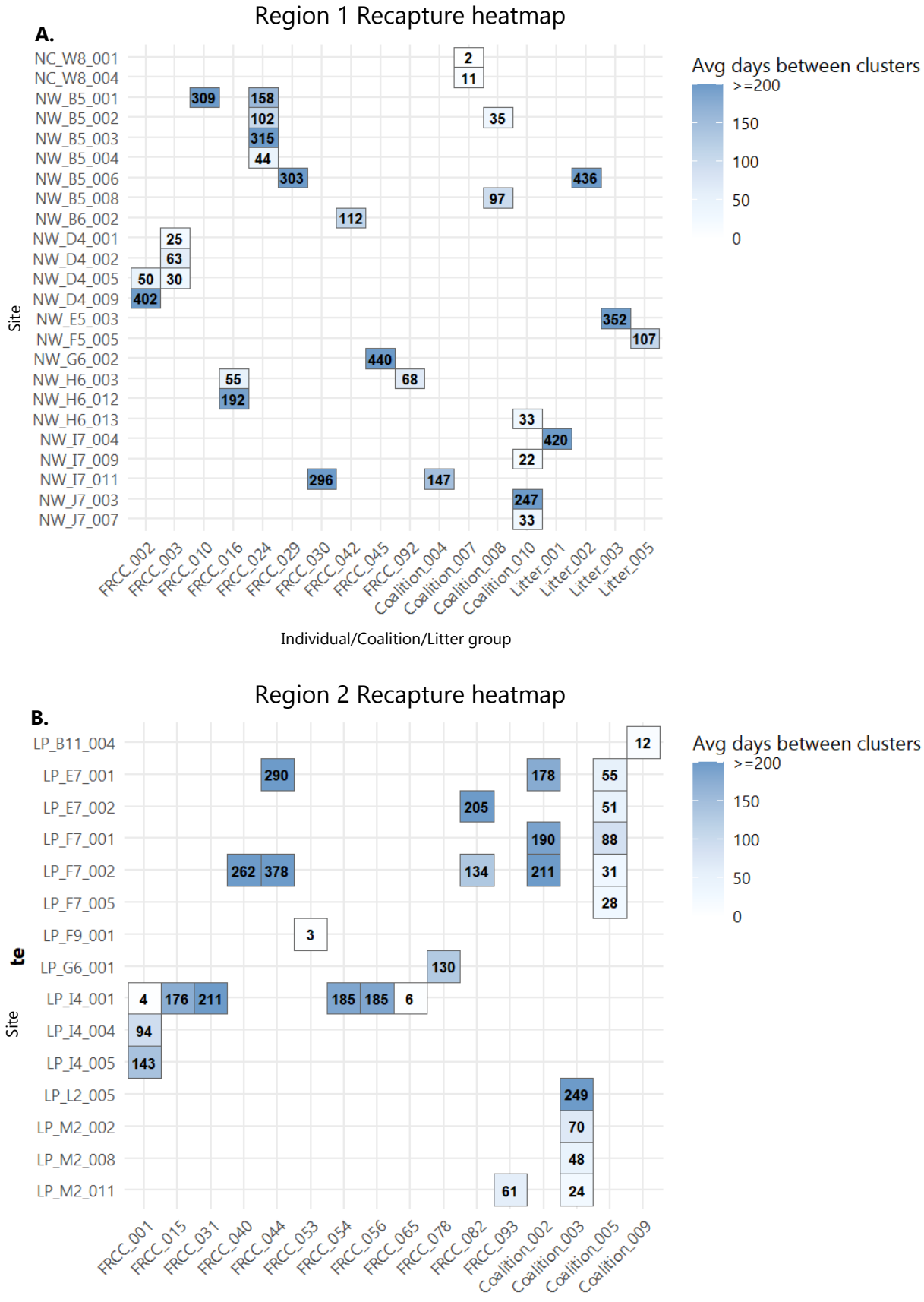

***Fig. 24**: Recapture heatmaps for Region 1 (**A**) and Region 2 (**B**). Light blue coloration is directly linked to low numbers inside the blocks, which indicate that the average days between a recapture of a specific individual at a specific site is low (i.e. fast recapture rate).*

***Table 8**: The image count of each predator captured on camera trap per region. Note that the high cheetah image count compared to the other predators is not necessarily because of more individual presence compared to other predators, but due to the camera site placement being aimed to capture cheetah over other predators.*

Predatory species	Avg. body weight (kg)	Region 1 (NC + NW)	Region 2 (NW + LP)	Total
Afro-Asiatic wildcat (*Felis lybica*)	5	640	859	1,499
Black-backed jackal (*C. mesomelas*)	9.5	11,267	11,077	22,344
Brown hyaena (*P. brunnea)*	42	1,071	3,127	4,198
Caracal (*C. caracal*)	13	1,105	1,003	2,108
Cheetah (*A. jubatus jubatus*)	**48**	**7,313**	**8,570**	**15,883**
Leopard (*P. pardus*)	46.5	287	2,130	2,417
Serval (*Leptailurus serval*)	13	35	102	137
Spotted hyaena (*Crocuta crocuta*)	63	35	844	879
Wild dog (*L. pictus*)	27	-	47	47

4.5.2 Activity patterns

The more restricted movement of Region 2 is also reflected in the cheetahs' activity patterns. Activity patterns show when cheetah are active over the 24-hour cycle estimated from the times at which individuals are recorded (by means of camera trap timestamp). Since activity is a function of time, it is usually visualised as circular density plots (0 to 2π radians) to visualize the continuation of days. For easier readability, non-circular graphs (Fig. 25) were created with both a radian axis (top) as well as a more intuitive 0-to-24-hour axis (bottom). The y-axis shows density, which represents the proportion (or probability) of clusters occurring at each time of day. A higher density value at a given time means that proportionally more clusters fall at that hour.

These activity graphs help us to identify main activity windows (e.g., nocturnal, crepuscular, diurnal) and to compare temporal niches between sexes, or regions. Region 1 exhibited a single, broad peak (Fig. 25A: density = 0.10 between 02:30–05:30 h), whereas Region 2 showed two higher, narrow density peaks (Fig. 25B: density = 0.125 between 04:00–05:00 h; density = 0.11 between 15:30–17:00 h). The fragmentation of Region 2 likely concentrates individuals' movement to a limited number of areas, increasing their detection frequency at specific

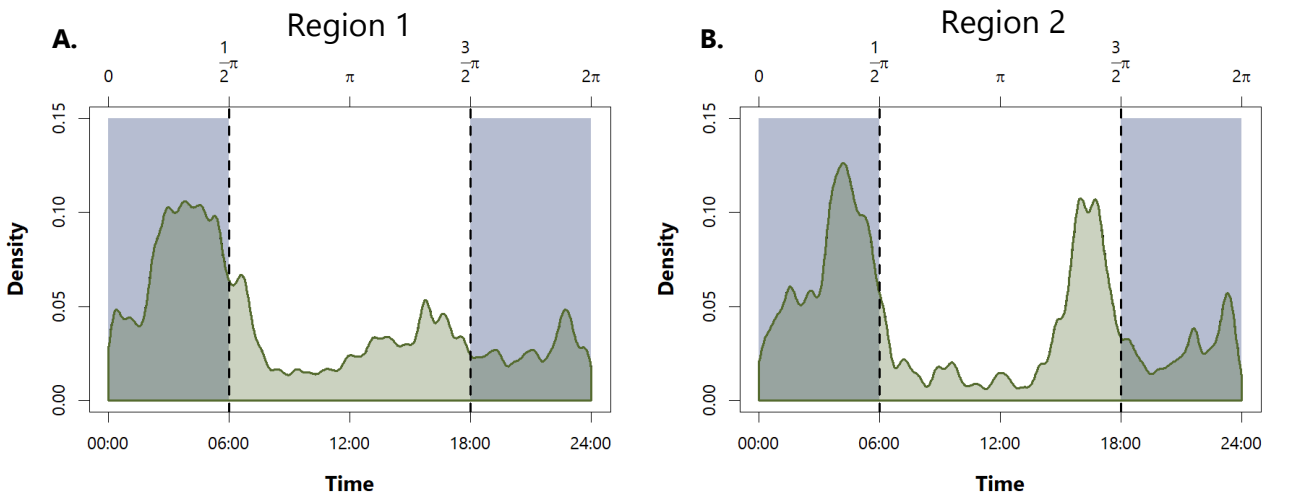

***Fig. 25**: Activity pattern graphs for Region 1 (A) and Region 2 (B). The green area under the curve equals to one to create proportional activity for each hour.*

camera sites. With slightly fewer distinct groups and a higher revisiting rate by the same individuals, these repeated detections amplify the apparent height of the activity peaks.

Sex-specific patterns highlighted more female activity peaks during the day compared to males for both regions (Fig. 26). Region 1 females (N = 11) peaked sharply at dawn (Fig. 26A: density = 0.29 at 07:00h), while Region 2 females (N = 12) showed multiple moderate peaks (Fig. 26B: density = 0.15) throughout the 24hours. Male activity remained more uniform across both regions ($N_{Region1}$ = 15, $N_{Region2}$ = 13), resembling the pooled sex patterns of Fig. 25. Females with litters need to hunt opportunistically to meet their demand for food. This can push them to be active throughout the day. Females without litters are solitary, often having a wider range to search for prey and potential mates. In contrast, male coalitions defend stable territories, actively using sentinel points (whereas females use them re-actively) which reduces their need for wide-ranging movement, slowing down their activity pattern and "smoothing out" their activity peaks over time [20][59].

Overall, cheetah showed a preference for crepuscular activity **but also exhibited relatively high peaks during both daytime and nighttime periods**. This temporal flexibility likely stems from the diverse activity rhythms of key prey species such as springbuck, steenbok, hartebeest, and impala, which are active across 24h cycle, as well as the opportunistic hunting demands of mothers provisioning for their young at any time of day. Understanding these activity patterns is important for free-roaming cheetah, as it informs human-wildlife conflict mitigation (e.g., timing livestock or game protection), and supports habitat management to align predator-prey dynamics in fragmented landscapes.

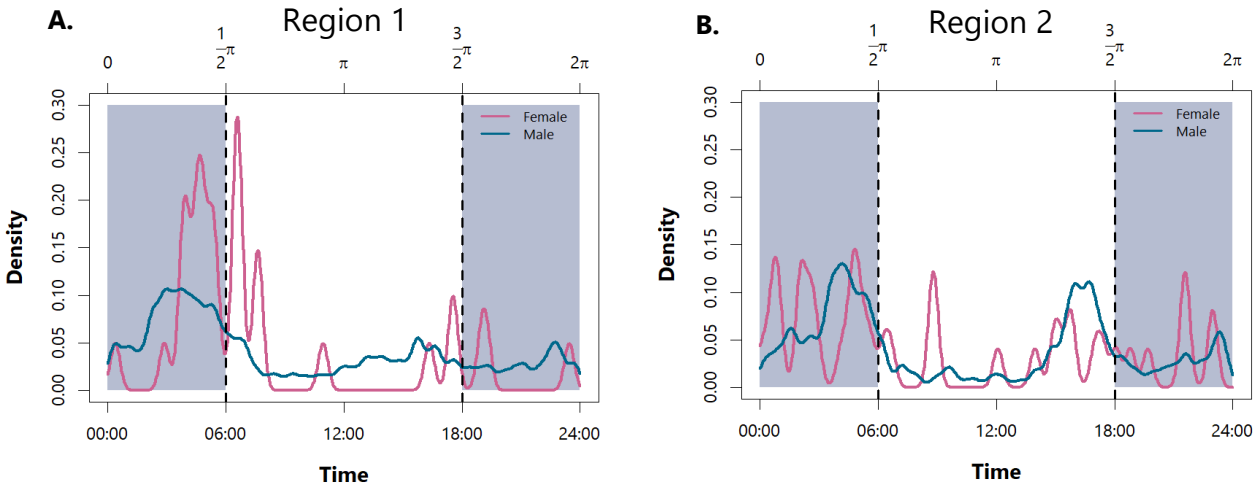

***Fig. 26**: Sex-specific activity patterns for Region 1 (A) and Region 2 (B). Male coalitions and mothers with cubs are counted as one individual.*

4.6 Subjective landowner observations versus objective presence data

The questionnaire provided a different data type compared to our objective methods. While camera traps, satellite collars, and DNA analysis yield factual/objective evidence of cheetah presence, questionnaire responses rely on landowners' perceptions and tolerance of predators, producing more perceived/subjective data.

4.6.1 Presence

Across 175 grids, data collected was either subjective, objective or both (Table 9). In total,147 grids had subjective survey responses, and 110 had objective data. Of the 147 grids surveyed with a questionnaire, cheetah presence was perceived across 85 grids (58%; Fig. 27A), although, the objective data only confirmed cheetah presence in 75 grids (68%; Fig. 27B).

To evaluate both datatypes properly, all grids with both perceived and effort for proven cheetah presence (N=77) data were compared (Table 9). Of these grids, objective evidence supported landowners' claims in 53 grids (69%). In the remaining 31% of grids (24 out of 77) where landowners reported cheetah presence, but objective data found none, perceptions differed from reality. This mismatch suggests false positives mostly driven by past (>5 years ago) personal or familial cheetah sightings, conflict with predators in general and people's tolerance.

Table 9*: This table shows the respondent and grid count for both the subjective (questionnaire) and objective (confirmed cheetah based on camera trap, telemetry, sightings with proof, DCA removals and faecal data). The numbers with which the percentages have been calculated are presented between the brackets for clarity.*

Metric	Sample size (N)	Percentage
Respondents	299	-
Grids subjective data	147	-
Grids objective data	110	-
Grids subjective + objective data	84	
Respondents reporting presence	189	63% (189/299)
Grids subjective presence	85	58% (85/147)
Grids objective presence	75	68% (75/110)
Grids subjective presence + objective data collection	77	92% (77/84)
Grids subjective presence with proof of cheetah presence	53	69% (53/77)
Respondents reporting presence and conflict	35	19% (35/189)
Grids subjective conflict	30	35% (30/85)
Grids subjective conflict + objective data collection	28	36% (28/77)
Grids subjective conflict + objective presence	21	27% (21/77)

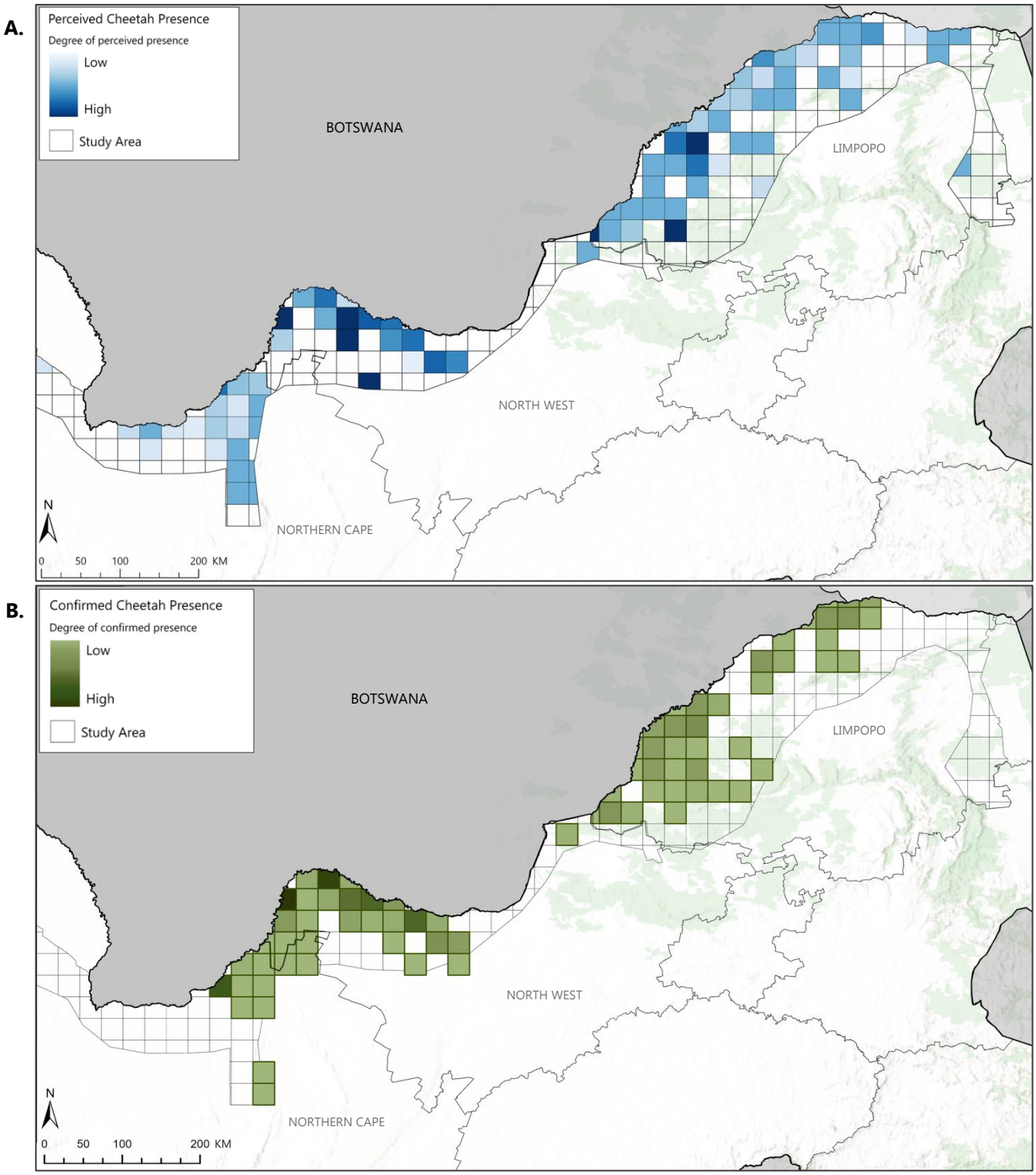

***Fig. 27**: Cheetah presence as indicated in (**A**) questionnaires and (**B**) confirmed with camera traps, mortalities, collars, scat collection and or video/photo evidence from landowners. The low and high degree of perceived presence (**A**) is based on the number of respondents in the grid that mentioned cheetah presence, compared to the total number of respondents in that same grid. The low and high degree of confirmation (**B**) is based on how many different types of data (1 – 5) have confirmed cheetah presence.*

The total area of confirmed cheetah presence was estimated by combining all 25 x 25 km QDGC in which cheetah occurrence was verified. Using this approach, **confirmed presence extended across ~ 21,875 km^2 in Region 1 and ~25,000 km^2 in Region 2.**

4.6.2 Conflict

Within the questionnaire, 189 (63%) out of 299 respondents within the study reported cheetah presence, with 35 farmers (19%) also reporting conflict with cheetah specifically across 30 different grids (Fig. 28). Conflict was determined on a low to high scale using conflict reports (yes/no) in the questionnaire, where the proportion per grid equals the number of participants reporting conflict divided by the total responses in the grid.

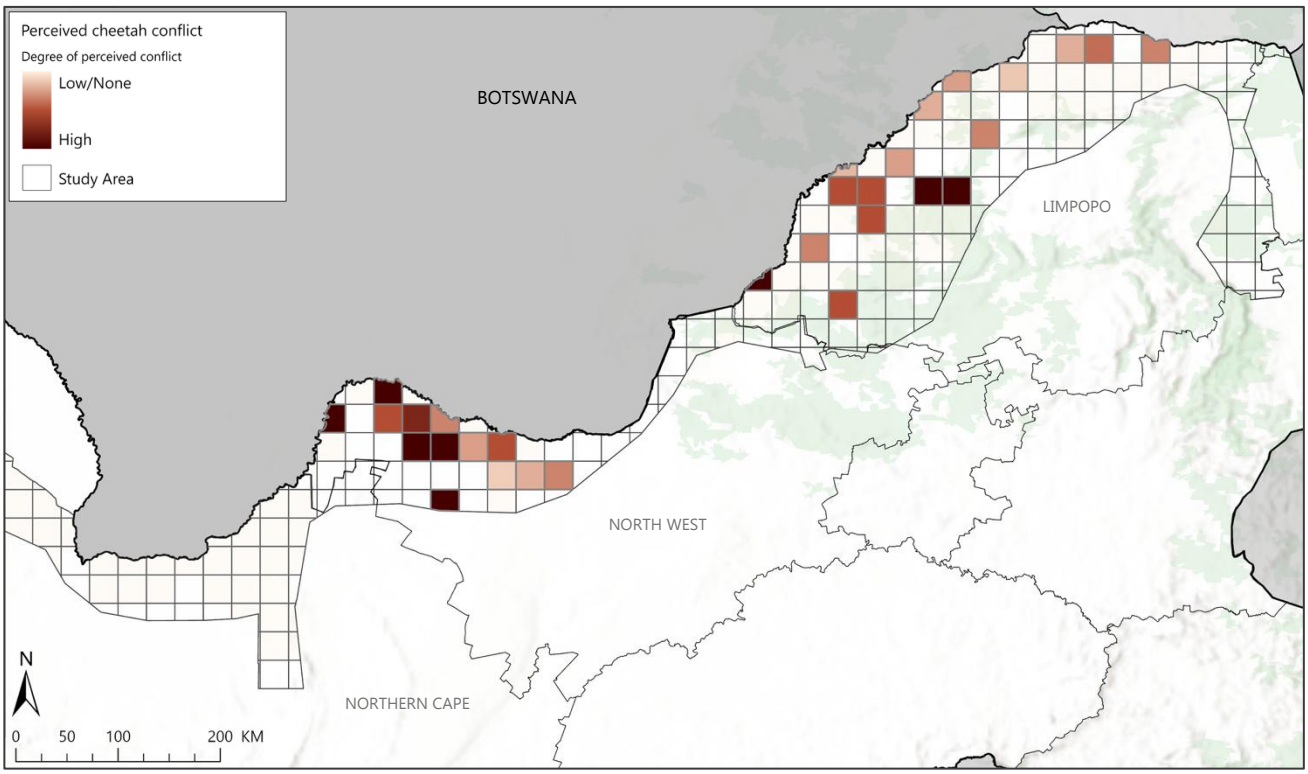

***Fig. 28**: All grids in which participants have mentioned cheetah presence together with cheetah conflict. The fully transparent grids have not reported any cheetah presence.*

Pearson's correlation analysis across 21 out of the 30 grids with additional camera trap presence, was executed to check whether a fast recapture rate (i.e. restricted movement; section 4.5.1) increases this human-cheetah conflict (Fig. 29). The Pearson correlation coefficient (r) measures the strength and direction of a relationship between two variables. It ranges from -1 to +1, with values near +1 meaning a strong positive relationship, and values near -1 meaning a strong negative relationship. Values near 0 mean little or no relationship [77]. Additionally, the analysis will provide a significance (p) value that indicates how likely the observed outcome was influenced by chance rather than facts. A small p-value means that the observed pattern is unlikely to be due to random chance. Therefore, true significant differences are obtained with low p-values (i.e. <0.05, which means that there is less than a 5% probability of seeing this result due to chance) [30].

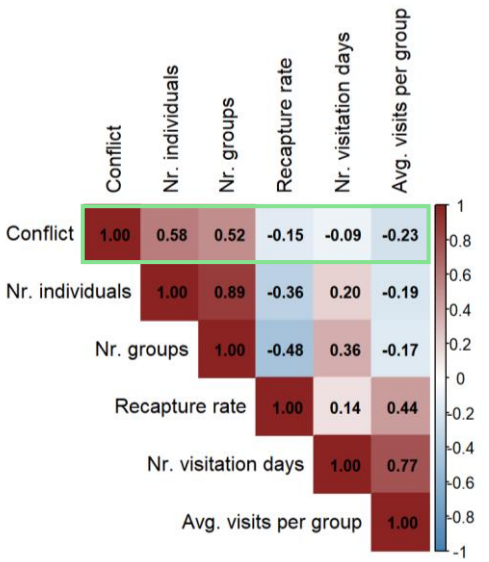

***Fig. 29**: Correlation matrix showing the effects of individuals, groups, recapture rate, visitation days and average visits per group on perceived conflict (green outline). Values <0 indicate a negative effect on conflict (e.g. high recapture, less conflict), while >0 values indicate a positive*

The analysis revealed that cheetah abundance (total individuals per grid) was the primary driver of perceived human-cheetah conflict (r = 0.58; p<0.01), followed by the number of groups (r = 0.52; p<0.05). Recapture rate, the number of days between a cheetah capture on camera within a grid, and average visits captured on camera within a grid did not have any significant effects on the

perceived conflict. Thus, increased perceived conflict is not related to how often/long a cheetah is present on a farm, but it is more related to the actual number of individuals being present in the area regardless of their actions (e.g. passing through, resting, hunting).

For each grid with reported cheetah conflict, dominant land use types (agriculture, ecotourism, livestock, game, or other) were identified based on the primary cover within each cell. Whenever a grid cell had equal mixes of multiple land use types, all types present were counted. High perceived conflict occurred exclusively in game/livestock-dominated grids (9 grids total), while low-to-medium conflict spanned 21 grids across varied land uses.

4.6.3 Tolerance

Tolerance (i.e. the willingness or ability of individuals or communities to deal with potential costs, both mental, physical and financial, from living alongside wildlife) and conflict are often considered closely linked to one another. To test whether this is the case within the census, tolerance was similarly scaled as the conflict (low to high) based on five questions assessing attitudes toward larger predators in general (Fig. 30).

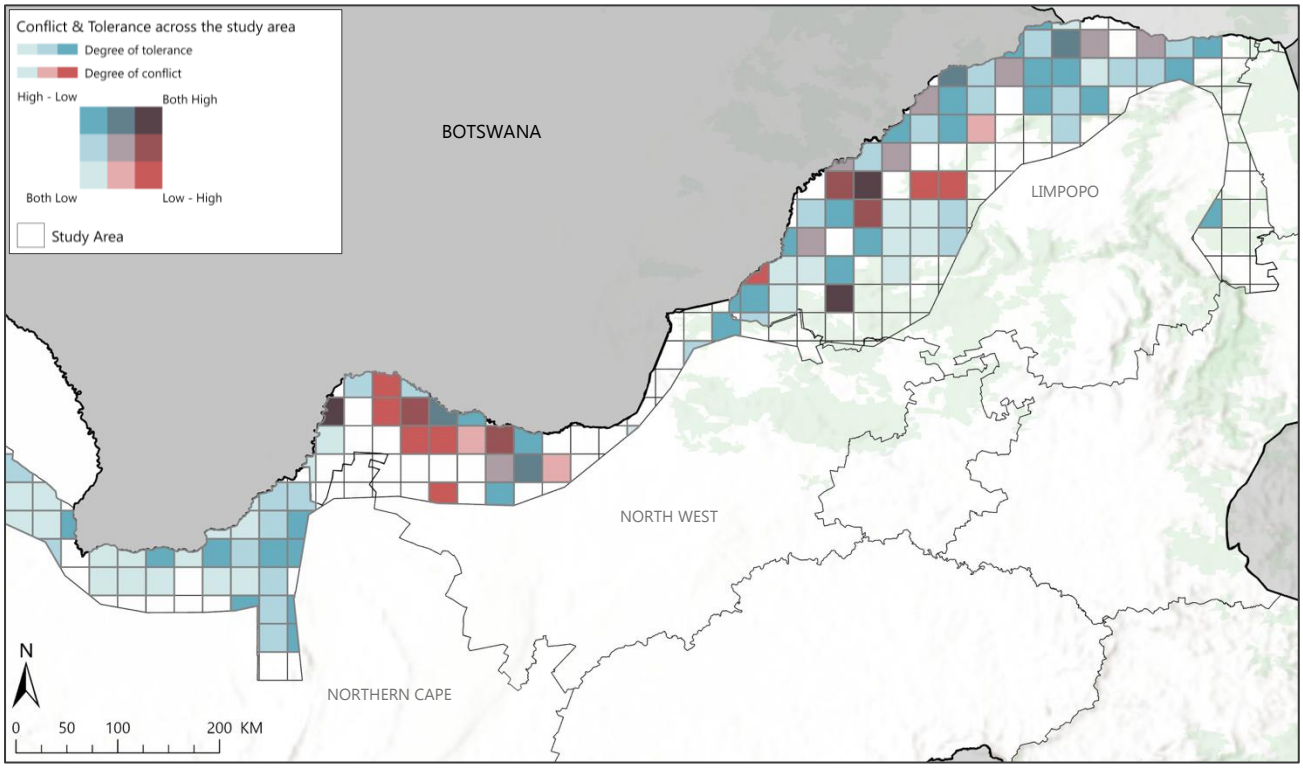

***Fig. 30**: A perceived conflict and tolerance map. The map consists of nine different colours, ranking from low conflict and low tolerance (light blue) to high conflict and high tolerance (dark red). This map indicates people's tolerance while taking conflict occurrences into account.*

Almost a third (31%) of the conflict grids showing low conflict paired with high tolerance, 10% exhibited very high conflict and very low tolerance, 25% reported both low conflict and low tolerance, and 3% endured high conflict yet remained highly tolerant. This pattern reveals that conflict and tolerance, while closely related, are distinct; high conflict does not inevitably result in low tolerance. For identifying new conservation areas or cheetah corridors, both conflict and tolerance must therefore be considered. Farmers in South Africa would like to be supported in ways that increase their tolerance towards predators.

From a conservation perspective, areas with high tolerance, even amidst high conflict, may present opportunities for the development of functional corridors that facilitate cheetah movement. Importantly, these findings highlight that range maps should extend beyond ecological suitability alone to incorporate socio-ecological

factors such as conflict intensity and tolerance, as areas with high conflict may ultimately represent low habitat viability for sustaining cheetah populations despite otherwise suitable environmental conditions.

Differences in conflict perception and tolerance are likely shaped by cultural values, education, financial constraints, and access to mitigation interventions. Within the questionnaire, 227 responses were submitted from farmers experiencing yearly unwanted predator interactions, of which 75% mentions their tolerance towards predators will increase when there is sufficient support. These participants also responded to questions specifically aimed at how they would like to be supported. This open-ended question resulted in seven distinct types of support requested (Table 10). The most frequently requested form of support was financial assistance. Importantly, this was not limited to direct financial aid, but also compensation for lost livestock or game, assistance with veterinary costs following predator attacks, and support for anti-poaching efforts, since poaching combined with depredation can further intensify hardship. Although financial aid is difficult to provide without government involvement, some NGOs, such as COT, are already contributing through research support, trapping and relocation assistance, and training guard dogs.

***Table 10**: Questionnaire responses of 227 participants on how they would like to be supported to increase their tolerance towards free-roaming predators.*

Support requests	Region 1	Region 2	Total
Financial support	94	47	141
Predator trapping and relocation assistance	26	21	47
Predator based research and monitoring	3	12	15
Hunting permits	3	7	10
Guard dogs	1	5	6
Funding to place and maintain fencing	0	5	5
Education on coexistence with predators	0	3	3
			227

4.7 Genetics

4.7.1 Scat DNA sampling

A total of 106 cheetah host DNA samples extracted by Jonah Ventures were analysed by the University of California Davis Veterinary Genetics Laboratory for relatedness. After microsatellite genotyping, 26 individuals (N_{female} = 6; N_{male} = 20) were identified ($N_{Region1}$ = 18; $N_{Region2}$ = 8) from 82 samples, while 24 samples (22%) yielded no result. These 'failed' samples could be due to either low-quality, degraded DNA or environmental contamination [45]. Of the 26 individuals, seven individuals were completely unrelated, while the remaining individuals formed six unrelated lineage groups. Further in-depth analysis for relatedness and inbreeding, although possible, was not done due to the relatively small sample size and low-quality DNA samples (extracted from scat) as this could provide inconsistent results [71]. Thus, it was not possible to confidently determine the degree of relatedness among individuals. Despite this, the spatial distribution of these lineages across the study area (Fig. 31 and Fig. 32) provided valuable insight into movement patterns and landscape use.

Region 1 distribution indicated a relatively high degree of connectivity, despite the absence of confirmed relationship degree. Multiple lineage groups are detected across a wide geographic extent, with movement patterns suggesting that some individuals transverse considerable distances between sampling locations. Importantly, several distinct lineage groups converge on the same sentinel sites, where repeated detections occur over time. This provides strong evidence of functional connectivity and that individuals are not always spatially restricted.

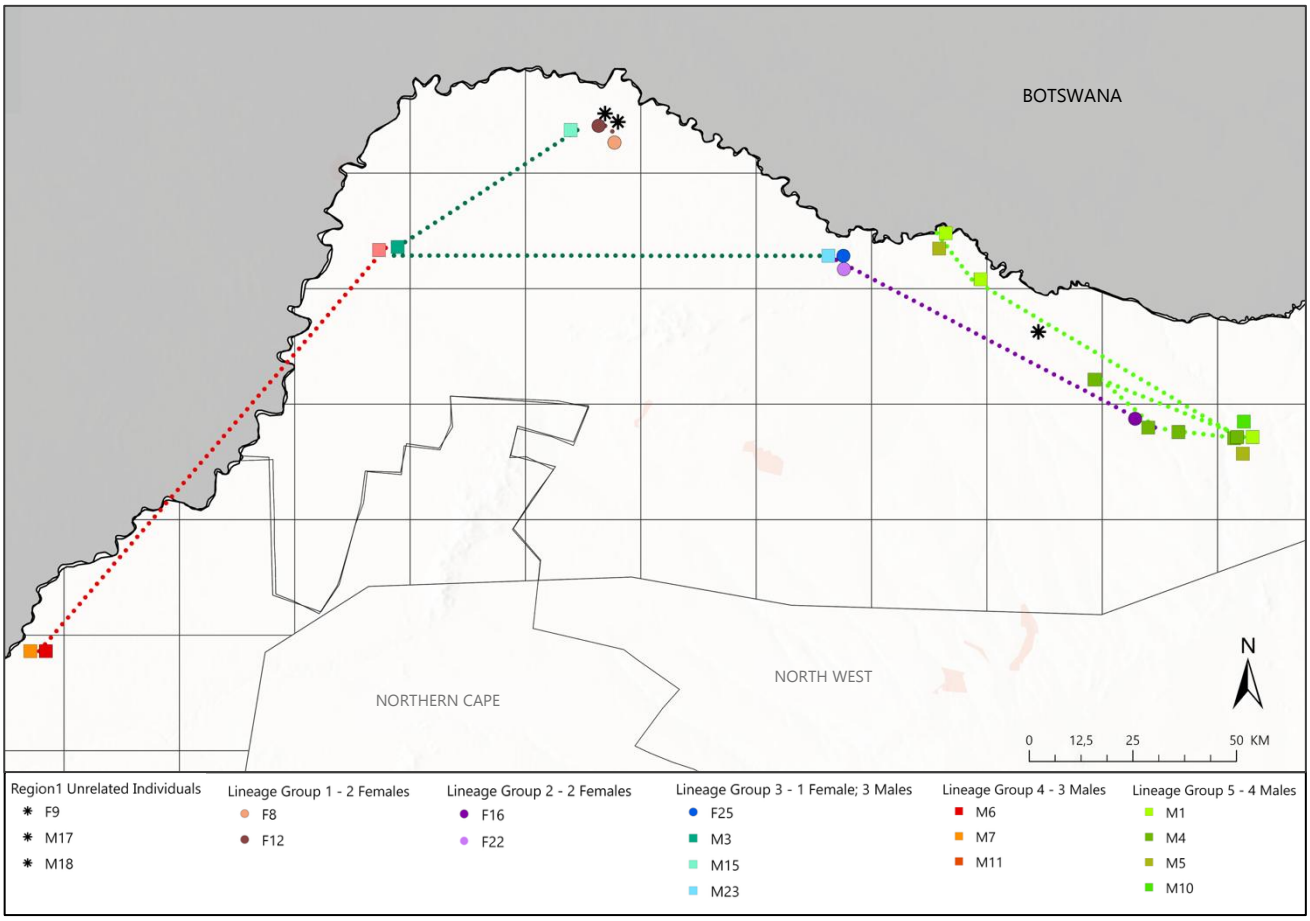

***Fig. 31**: Distribution of genetically identified cheetah lineages in Region 1. Each colour within a region denotes a unique genetic lineage, with connecting lines illustrating inferred movement. Lineage assignments are based on genetic differentiation and do not represent degree of kinship or relatedness.*

In Region 2, the distribution of lineage groups suggested spatially constrained movement, although connectivity is still evident. Individuals show movement across the landscape, with some convergence occurring at shared

sentinel sites. However, compared to Region 1, the spatial spread of detections was more limited. It is important to note that sampling effort was considerably lower than in Region 1, largely due to logistical constraints that included weather conditions and limited habitat accessibility. As such, the observed patterns likely underrepresent the true extent of movement and site use.

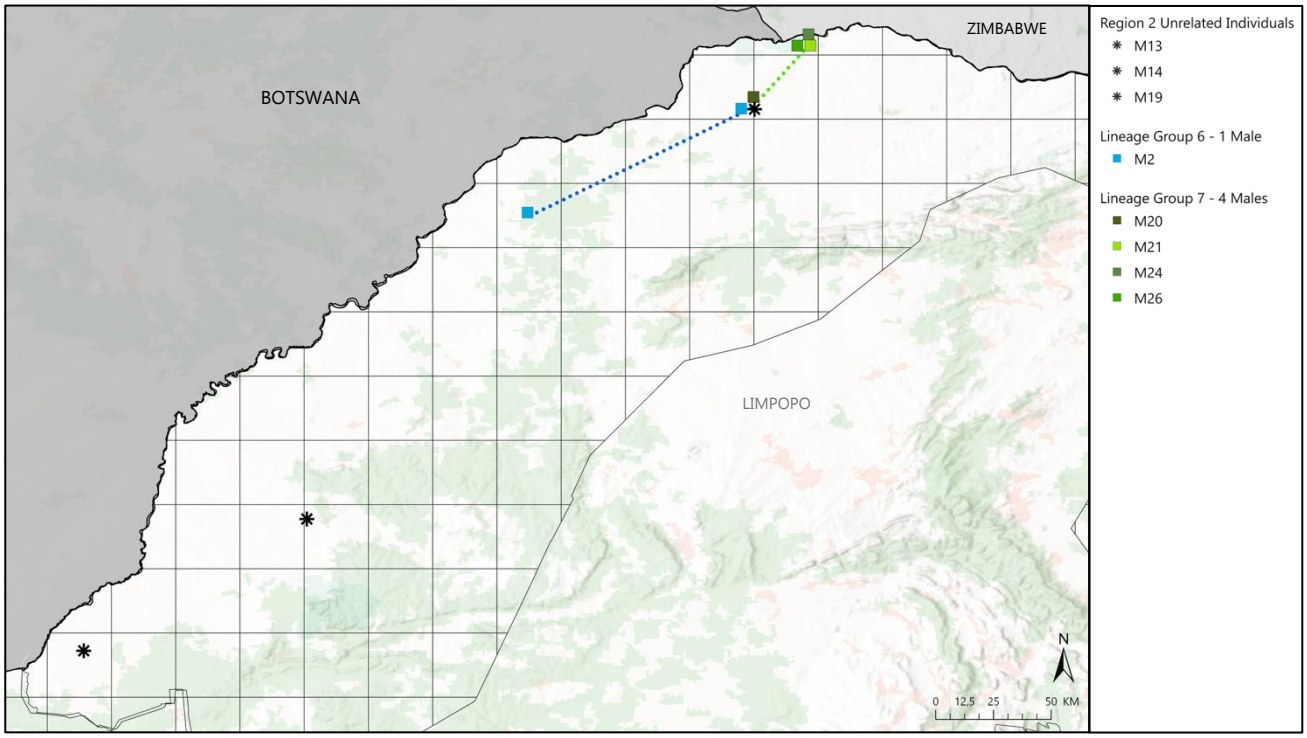

***Fig. 32**: Distribution of genetically identified cheetah lineages in Region 2. Each colour within a region denotes a unique genetic lineage, with connecting lines illustrating inferred movement. Lineage assignments are based on genetic differentiation and do not represent degree of kinship or relatedness.*

An effort was made to integrate these genetic results derived from scat with camera trap detections; however, it was not possible to confidently match genetically identified individuals to those observed on camera. Besides a lone 2-male coalition (Lineage Group 4) in the Northern Cape, this uncertainty was further pronounced at sites where multiple cheetah were present. Consequently, genetic and camera trap datasets were interpreted independently, with any potential overlap considered suggestive rather than conclusive.

4.7.2 Blood/Skin sampling

Samples (N = 46) that were obtained from cheetah from within the two regions were analysed by the Unistel Medical Laboratory in Cape Town, South Africa (Appendix VII). DNA was extracted from whole blood, blood on Guthrie-cards and tissue using the E.Z.N.A. Blood DNA Mini Kit., although only 33 samples ($N_{Region1}$ = 11; $N_{Region2}$ = 22) were successfully amplified on the UML Feline Predator STR panel. Interpretation of Region 1 samples should however be approached with some caution due to the smaller sample size available. For this report, the diversity indices of the Number of Alleles (N_a), the Effective Number of Alleles (N_e), Heterozygosity (H_o and H_e), Fixation Index (F_{IS}) Inbreeding Coefficient and the Relatedness between individuals were summarized.

4.7.2.1 Allele Diversity

Number of Alleles (N_a)	Calculates how many variants are found at each genetic marker within the dataset.		
	Interpretation	*Value*	*Favourable Outcome*
	Higher N_a (2 – 5): indicates a greater variety of genetic options within the group. **Lower N_a (1.5 – 2):** suggests a limited number of alleles, which can be attributed to previous population declines, small population size and isolation or limited gene flow. **N_a values lower than 1.5:** can be seen as a cause of concern.	Identifies uncommon alleles that might not significantly influence heterozygosity (the possession of two different alleles of a particular gene). Aids in monitoring the decline of genetic diversity over time. Valuable for pinpointed populations that may have experienced bottlenecks. Crucial for assessing long-term adaptive capacity, even when the present health of the population appears stable.	A higher number of alleles per locus is generally favourable, with values above 4 – 6 indicating good genetic diversity, while very low values (<2) may suggest reduced variation or isolation.
Effective number of Alleles (N_e)	Estimation of how many alleles are effectively contributing to genetic diversity, considering how evenly allele frequencies are distributed.		
	Interpretation	*Value*	*Favourable Outcome*
	N_e close to the N_a value: alleles are evenly distributed. **N_e value lower than N_a:** a few alleles dominate the population. **N_e of 1.3 – 2.5:** adequate diversity. **N_e of 1.1 – 1.3**: caution is advised. **Very low N_e (<1.1)** cause for concern – reduced functional diversity, even if multiple alleles exist.	N_e is more informative than N_a for understanding real genetic diversity and is a strong indicator of population stability, genetic drift and long-term evolutionary potential.	Both N_a and N_e is low but values remain stable across loci and groups over time. If N_a is moderately high but N_e is very low – this would suggest some alleles although present, are being lost through genetic drift.

The Region 1 dataset exhibited moderate allelic diversity (Fig. 33), with a mean number of alleles (N_a = 4.16) and an effective mean number of alleles (N_e = 2.8). The Region 2 dataset also exhibited moderate allelic diversity, with a mean number of alleles (N_a = 4.8) and an effective number of alleles (N_e = 3.29). Both indicate a relatively even distribution of alleles across loci (the fixed physical location of a DNA sequence on a chromosome).

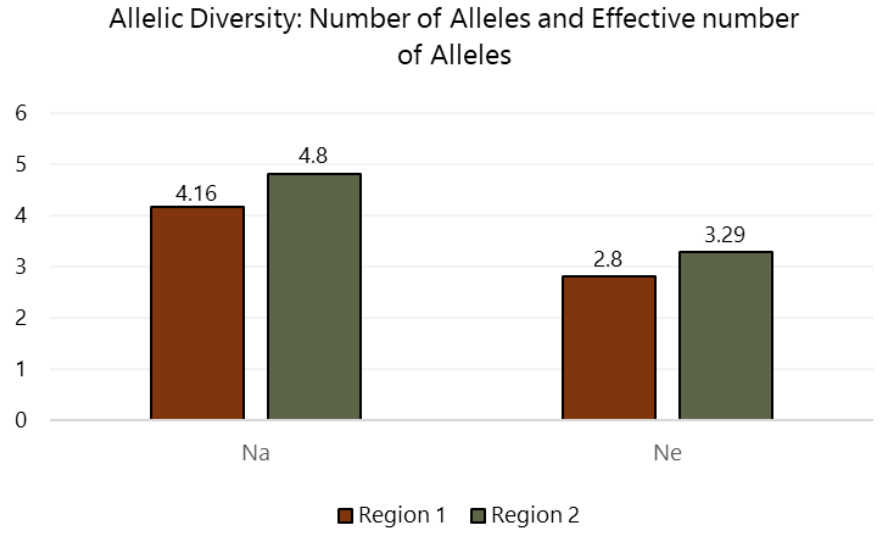

***Fig. 33**: The mean number of alleles (Na) and effective number of alleles (Ne) for both regions.*

4.7.2.2 Heterozygosity and Fixation Index

Heterozygosity (H_e and H_o)	Utilised to assess the degree of genetic diversity present within a population. **H_e: what is expected. H_o: what is observed**		
	Interpretation	*Value*	*Favourable Outcome*
	H_o and H_e are close in value: indicates healthy genetic mixing. **H_o lower than H_e:** possible inbreeding or separation into sub-groups. **Values between 0.3 – 0.5:** no cause for concern. **Values below 0.3**: caution. **Values below 0.25:** concerning.	Higher genetic variation generally indicates better adaptability and resilience to diseases and environmental changes.	Higher values are considered more favourabe, however, within cheetah groups, values are expected to be lower than in many species.
Fixation Index (F_{IS})	Measuring whether animals are more genetically similar than expected which would indicate whether animals within a sub-population is breeding mostly among themselves.		
	Interpretation	*Value*	*Favourable Outcome*
	Values close to 0 (0-0.10): indicates that individuals within the group are likely mating at random (showing no significant signs of inbreeding). **High positive value (0.1–0.15):** suggest there is less diversity observed within the group than expected. *Values greater than 0.15 is a cause for concern.* **Negative value**: indicates more diversity within the group than expected, which may rise from admixture between groups or sampling bias within the dataset.	Positive values may indicate inbreeding, restricted mate choice or hidden population subdivision.	Values close to 0 are ideal.

In Region 1, observed heterozygosity (H_o = 0.580) slightly exceeded expected heterozygosity (H_e = 0.568), resulting in a mildly negative inbreeding coefficient (F_{IS} = -0.034; Fig. 34). This pattern suggests a small excess of heterozygotes and indicates little evidence of widespread inbreeding within the population. In Region 2, observed heterozygosity (H_o = 0.527) was lower than expected heterozygosity (H_e = 0.639), resulting in a positive inbreeding coefficient (F_{IS} = 0.155).

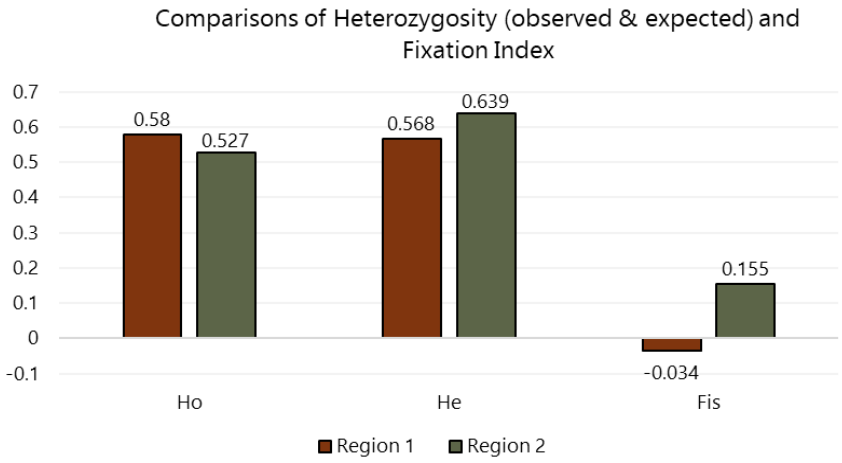

***Fig. 34**: The observed heterozygosity (Ho) and expected heterozygosity (He) for both regions.*

This pattern within the Region 2 population indicates a deficit of heterozygotes, suggesting possible inbreeding, population substructure (the division of a larger population into smaller, distinct, and often genetically differentiated groups due to non-random mating, geographic barriers or limited migration), or the presence of highly related individuals within the sampled population.

4.7.2.3 Inbreeding coefficients and relatedness estimates

Relatedness	Measures how genetically similar two animals are. It estimates whether two animals are likely to be close relatives or not.		
	Interpretation	*Value*	*Favourable Outcome*
	High Values (0.1 – 0.2): likely close family (siblings, parent-offspring). **Values close to 0 (-0.05 – 0.1):** Unrelated animals **Values larger than 0.2** can be seen as cause for concern.	If many animals within a group share close genetic ties, it may indicate restricted movement, limited mating options and reduced population size.	Most animals should not be closely related. A certain level of relatedness is typical, but a population primarily composed of close relatives could face potential risks.
Inbreeding coefficient	Estimates how likely it is that an animal inherited the same genetic material from both parents, i.e., how closely related were the animals' parents to each other.		
	Interpretation	*Value*	*Favourable Outcome*
	Values close to 0 (0-0.1): parents were not closely related. **Higher values (0.1-0.15):** increased level of inbreeding. **Values larger than 0.15** should be seen as a cause for concern.	High inbreeding can reduce fertility, survival, and disease resistance over time.	Low values are best. While some inbreeding is anticipated because of historical population declines, excessively high levels could be concerning, particularly if they continue to rise.

Pairwise relatedness was estimated using the Trio Maximum Likelihood (TrioML) estimator in the software package Co-ancestry, which is used to estimate the degree of kinship between individuals based on molecular data such as microsatellites (Fig. 35A). Additionally, the Dyadic Maximum Likelihood (DyadML) estimator was also utilised. This is a statistical approach used to estimate the pairwise relatedness or kinship coefficient between two individuals (a dyad) based on their microsatellite genotypes, without relying on a pre-existing pedigree. Both estimators are widely regarded as two of the most accurate estimators when dealing with inbred populations, small sample sizes or data containing genotyping errors [38]. For individual inbreeding coefficients, Ritland estimators (Ritland and LynchRD) are widely used to estimate the distribution of inbreeding in natural populations where pedigrees are unknown as well as estimate how related two individuals are and how inbred a single individual is (Fig. 35B,[34]).

Although relatedness and inbreeding are closely linked, they capture different aspects of population genetic structure. Relatedness reflects the average degree of kinship among individuals within a population, whereas inbreeding measures the probability that an individual has inherited identical alleles from related parents [10]. As a result, populations may exhibit elevated relatedness without strong inbreeding if close relatives are not mating, or elevated inbreeding without uniformly high relatedness if related matings occur within particular subgroups.

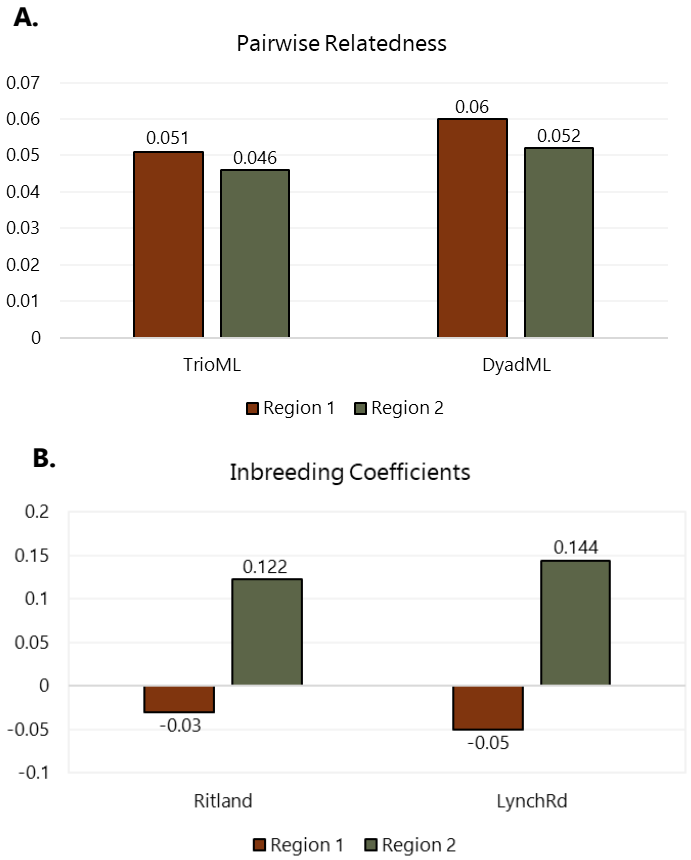

***Fig. 35**: The pairwise relatedness, kindship among individuals within a population, (**A**) and inbreeding coefficient, inheritance of identical alleles, (**B**) for both regions. The negative inbreeding value for Region 1 (LynchRd of -0.0046) does not indicate outbreeding, but it means that the estimate is slightly below zero because the individual appears to be a bit less related to the reference population than expected.*

Mean relatedness values were low in both regions, indicating that most sampled individuals were not closely related. Region 2 exhibited lower relatedness estimates (TrioML = 0.046; DyadML = 0.052), consistent with a broader genetic base and greater mixing among individuals. However, inbreeding coefficients for this region were moderately elevated (Ritland = 0.122; Lynch & Ritland = 0.144), suggesting some mating among related individuals or the presence of population substructure.

Region 1 displayed similarly low relatedness estimates (TrioML = 0.051; DyadML = 0.060), indicating limited overall kinship among individuals supporting the interpretation of a relatively well-mixed population. Inbreeding estimates for Region 1 were near negative (Ritland = -0.030; LynchRd = -0.050), indicating little evidence of close kin breeding. Together, these patterns suggest that while Region 1 individuals may share ancestry due to localised founder effects and the presence of family groups, breeding practices appear to limit inbreeding. Conversely, Region 2 reflects a broader genetic base but exhibits signs consistent with substructure or restricted gene flow that may increase inbreeding risk.

4.7.3 Relationship between the two regions

Further analysis (population genetic distance, identity, gene flow and the Bayesian package called STRUCTURE) was undertaken to assess the overall relationship between the two regions. These results indicated that there was movement between the regions, resulting in only moderate genetic differences. Overall, the population appeared to be broadly connected, relative to genetic distances, rather than strongly isolated.

Taken together, these findings indicate that the sampled cheetah form a genetically connected population with localised family groups but with no strong regional isolation as of yet. Close relatives occur within the population, although individuals from different areas share ancestry and contribute to a shared gene pool. Although genetic analysis indicates ongoing or recent activity between the two regions, ecological evidence suggests that barriers such as increased human population density are becoming more restrictive. Parapatric structuring is observed when neighbouring populations are separated by a semi-permeable barrier, that limits, but does not completely prevent, dispersal [94]. While full speciation, the process by which a single population splits into two groups that become genetically distinct, has not occurred, the system reflects conditions consistent with parapatric differentiation driven by reduced but not entirely absent, gene flow [39].

This emerging isolation is of particular concern given the small number of mature individuals (N_{Adults} = 83; $N_{Region1}$ = 33; $N_{Region2}$ = 50) identified during the census. Small and fragmented populations are especially vulnerable to genetic drift, inbreeding, and the gradual loss of genetic diversity, all of which may reduce long-term adaptive potential and population persistence. Conservation genetics frameworks proposed the "100/1000 rule" for low breeding mammals, suggesting that a minimum effective population size of approximately 100 individuals is required to limit short-term inbreeding effects, while 1000 or more individuals are necessary to maintain long-term evolutionary potential [69].

5. Policy and Management in South Africa

South Africa's northern regions face ongoing human-predator conflicts, as a large variety of predators occasionally prey on game or livestock, threatening farmers' livelihoods [92]. Therefore, balancing cheetah conservation within a human dominated system requires robust and clear policies, yet South Africa's policy framework remains as a mostly outdated, patchwork of rules that are often challenging to navigate. This section in the report briefly reviews past and current predator management related policies, rules, farmer interventions, attitudes, and principles for adaptive management to improve coexistence.

Both in the past and present, the black-backed jackal (*C. mesomelas*) and caracal (*C. caracal*) are perceived as high-conflict animals due to large, abundant populations compared to scarcer large predators such as leopard and cheetah [90]. This resulted in often lethal predator control with strong government backing. A very popular method was the rise of hunting clubs. In the 1960s, subsidized hunting clubs were so successful that the government believed that the depredation problems were under control by 1967 [18][7]. However, most hunting regulations are not target specific and often lead to lethal captures of other species, including large predators which naturally suppress the presence of mesopredators through competition for prey and occasional predation. With free-roaming lions eradicated from South Africa, African wild dogs nearly extinct, and cheetah numbers dwindling rapidly, this top-down control is lost. This results in the opposite effect where mesopredators like the jackal and caracal can once more roam unchecked [7][9].

This loss of larger predators, together with a rise in environmentalists, brought on a more restricted use of predator interventions. The 1970s brought restrictions via the Hazardous Substances Act 15 of 1973, which prevented the use of poison. By the early 1990s governmental subsidies decreased to a minimum [7], the Firearms Control Act 60 (2000) banned unethical devices like coyote getters to protect non-target species and CapeNature partnered with NGOs to phase out gin traps and night hunting [18]. By 1995, the National Problem Animal Policy Committee (NPAPC) imposed stricter rules, disfavouring the once popular hunting clubs, and creating more managed and regulated hunts. CapeNature followed in 1996, requiring consultations with animal rights groups, environmentalists, farmers, and academics for damage-causing animal control. Currently, no unified legal framework governs predator management. Instead, disconnected, outdated legislations create conflicts between groups, with administratively burdensome permit systems [23][44]. There is no coordinated conflict monitoring across provinces, with declining governmental aid for fencing or control [8][44].

With South African farmers mostly left to fend for themselves, while following an unclear set of rules and guidelines, frustration levels increase while tolerance towards predators decline. In the questionnaire (section 3.1), respondents were asked how predatory interactions affected their wellbeing. Almost 90% indicated that their wellbeing is strongly dependent on how well their farming is doing (i.e. livestock/game survival, maintenance and financial stability). However, 61% explicitly linked predator conflict to negative impacts on their farming practises and, consequently, on their overall wellbeing. Farmers employ diverse interventions, often favouring non-lethal options such as predator-proof/game fencing and livestock guard animals like dogs, llamas, alpacas, and donkeys [78][9]. Unfortunately, almost all participants reported receiving no governmental support, either financially or for mitigating/preventing conflict, resulting in farmers often opting for lethal methods to eradicate the problem.

Lethal methods are mostly used on larger predators (≥15 kgs) as they are often considered the main cause for stock losses on farmland. Currently the cheetah is one of the predators struggling the most with human persecution. The lethal methods oftentimes used on cheetah are not just a matter of budget or to prevent further livelihood losses, but also hinge on cultural traditions, ethics, and neighbours' perceptions and actions [92][78]. The high persecution rates have caused the cheetah to be marked as Vulnerable on the IUCN Red List. They have been on South Africa's Endangered Species List since 1970 and under CITES Appendix I protection since 1975 [4]. The Endangered listing is strictly for national/domestic protection of the species, while CITES Appendix I bans international commercial trade in cheetah or their parts, aiming to prevent overexploitation.

The combination of IUCN status and CITES listing, caused the cheetah to fall under the threatened or protected species (TOPS) regulations [4]. Under South Africa's National Environmental Management Biodiversity Act (NEMBA) [67], Section 101 outlines clear criminal offences related to TOPS. Here it states that a person may not carry out a restricted activity involving a specimen of a listed alien species, invasive species or TOPS species without a permit. However, permit holders can commit an offence by violating permit conditions, performing activities outside authorized terms, or allowing others to breach the rules. It is an offence to fraudulently alter, or forge permits, possess fake permits, or make false statements to obtain one.

A person convicted of an offence under NEMBA Section 101 faces imprisonment for up to five years, a fine which is equal to three times the commercial value of the specimen, up to R5 million, both, or, for second/subsequent offences, fines up to R10 million or imprisonment up to 10 years, or both. Fines are calculated using a standard formula in the Adjustment of Fines Act 1991 [65], which links fine amounts to imprisonment length via ratios set under the Magistrates' Courts Act 1944 [64. The maximum is either the prescribed amount under the Adjustment of Fines Act or three times the commercial value of the protected specimen, whichever is greater. Cheetah fall directly under these penalties [4][44]. Killing or harming one without a permit triggers Section 101 offences, with fines escalating to three times the cheetah's commercial value (typically between R100,000 – R150,000 within the cheetah trade) plus up to five years imprisonment.

Despite these TOPS regulations there has rarely been, if ever, a successful case brought against an offender for killing cheetah. These laws are for the protection of cheetah only but do not offer solutions or plans of compensation for the farmers whose stock is targeted. Therefore, harmful practices toward cheetah continue, as enforcement of these penalties rely on proven intent, rendering protections largely ineffective in practice. Near-certain evidence is needed that harm to the cheetah was deliberate, not accidental (e.g., mistaking a cheetah for another predator). This creates an evidentiary deadlock in which, regardless of the presence of regulations and penalties, environmentalists struggle to secure convictions without witnesses, ballistics, or confessions, while farmers claim "self-defence" or error. As a result, fines or jail time are rare despite frequent illegal killings. Therefore, cheetah populations continue to decline rapidly, with free-roaming numbers dwindling due to preventative or retaliatory killings, poaching, and habitat fragmentation, underscoring enforcement gaps along northern borders [4][9].

6. Census logistics and resources

Conservation projects are judged by the work on the ground. Success, however, often depends on the systems created prior. Due to the novelty as the first nation-wide free-roaming cheetah census in South Africa, there was little information to guide the census regarding budgeting or logistical needs. Thus, the initial planning for the census started approximately one year before fieldwork began in October 2022 and included in-depth discussions between the collaborators (ACC and COT) to understand what limitations and complications would be faced in attempting to survey to this spatial and logistical scale. A PhD student doing a double doctorate with the University of Groningen in the Netherlands and Stellenbosch University in South Africa, was brought on board to further expand the census planning with protocols and systems for data capture and analysis.

An incremental planning approach was adopted, limiting the spread of fieldwork to areas within confirmed cheetah range, allowing the team to field-test survey methodologies. This allowed for insight to time constraints, data collection practicalities and logistical requirements, which in turn informed the broader census design. During the testing phase of the census (Sept. 2022 – Oct. 2022), the initial team consisted of 1 x ACC staff/Student team with a company vehicle, and 2 x COT staff, each with company vehicles. A critical need for a scat detection dog was identified, especially in remote areas where the project could stall due to time constraints in finding appropriate sentinel sites to place cameras at. Detection dogs have been widely used in conservation projects to a high degree of success, even for animals occurring at low densities [41]. An Australian Cattle Dog (Blue Heeler), known for their sharp sense of smell, was sourced and trained by Rox Brummer at Green Dogs Conservation in Limpopo to specifically detect cheetah scat.

As the scope of the census increased, additional field team member (FTM) options were assessed in 2023. A local research organisation, Alldays Wildlife and Communities Research Centre (AWCRC) assisted by servicing camera sites on or near their area in Limpopo, COT employed another staff member, and ACC committed two more full time staff members to field work only. In total, there were seven field teams (three full time across the whole range and four in selected areas) (Fig. 36). Note that >100% FTM percentages do not reflect reduced workload, but include periods when fieldwork was not possible (e.g., holidays or restricted-access periods). During all available fieldwork periods, all teams operated at full capacity **relative to their role in the census**.

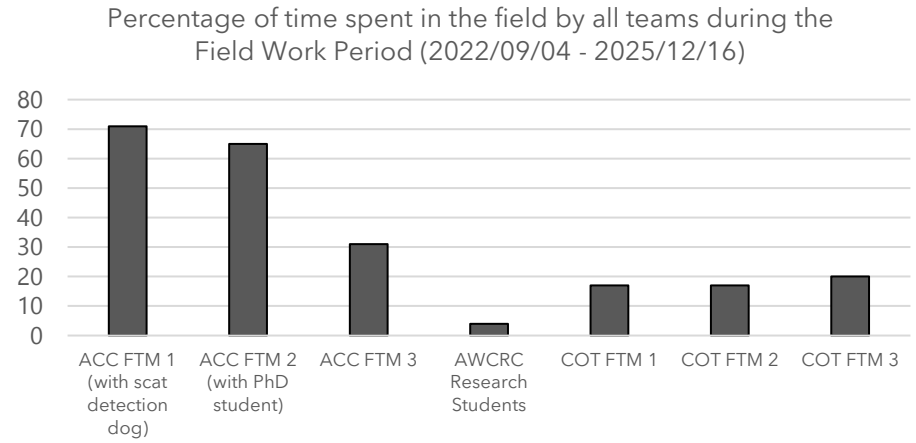

***Fig. 36**: Bar graph showing all field team members' (FTM) percentage of time spent on the ground doing camera trap and questionnaire fieldwork.*

To support the implementation of this census, a centralised project coordination and data management system was established at the Ashia Center in Paarl. This functioned as the operational core for distributing equipment, collection and processing of field data and the continuous resupply of material for the field teams to ensure efficiency was maintained in both data handling and resource allocation. Camera traps, along with camera necessities (e.g. SD Cards and batteries), were procured and labelled before being distributed to the field teams.

When servicing sites, teams would collect image data and, where possible, scat samples. After transferring the images to external hard drives, these drives would be couriered at regular intervals back to Ashia for storage, processing and uploading to TrapTagger. Similarly, any scat samples collected were sent along with the image data for storage until DNA analysis could commence. Equipment was replenished and distributed as needed, allowing the field teams to continue working efficiently across the study area.

Despite the above framework, external factors (e.g. weather and seasonal farmland practises) still influenced the consistency of the survey efforts. Heavy summer rain often limited accessibility, particularly in areas with poor road infrastructure, and would wash away any possible scat samples. Conversely, the winter hunting season in South Africa posed additional challenges, as many properties were inaccessible as property owners would be hesitant to allow field teams to service or place cameras during this period. The hesitancy was due to the safety risk to the field teams, or (primarily) the risk of the field teams creating unnecessary disturbance and noise while well-paying hunting clients are on the property. Throughout the whole study area, access during all seasons relied on the landowners' availability, which did delay planned surveys in certain areas.

Even with the extensive planning and coordination, most field operations ultimately depended on the reliability and condition of the vehicles used, especially given the remote locations travelled to during the census. Frequent wear and tear required regular servicing, and tyre replacements were common due to punctures and damage sustained in the field. Flat tyres were a routine occurrence and often had to be dealt with without any external assistance. For this reason, vehicles were fitted with reinforced sidewall-layered tyres for durability and for increased resistance to damage on gravel road. An additional layer of risk was the high presence of wildlife which required constant awareness to avoid collisions during periods of increased animal activity. Accidents still happened, and two vehicles sustained damage during the census period. One ACC vehicle was completely written off and required replacement, while the COT vehicle was damaged by a kudu, but was repairable.

Next to the constant and adaptive planning, significant financial investment was also required to sustain such a large operation. As the primary funder and coordinator of the FRCC, ACC carrying >60% of the total project expenses, excl. capital cost, of **USD 740,800** (R12.6m; Fig. 37, Fig. 38).

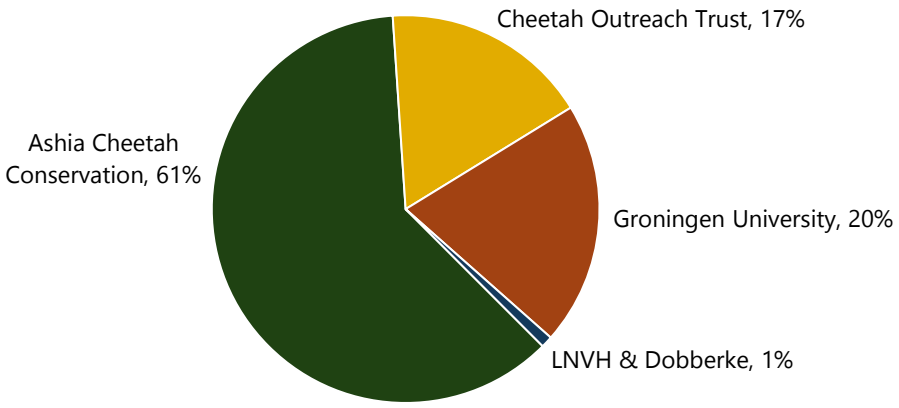

***Fig. 37**: All financial contributions of each collaborator in USD (1ZAR=17USD on average) The percentage contribution made by each collaborator grouped together for the distribution of the total spend USD 740,800.*

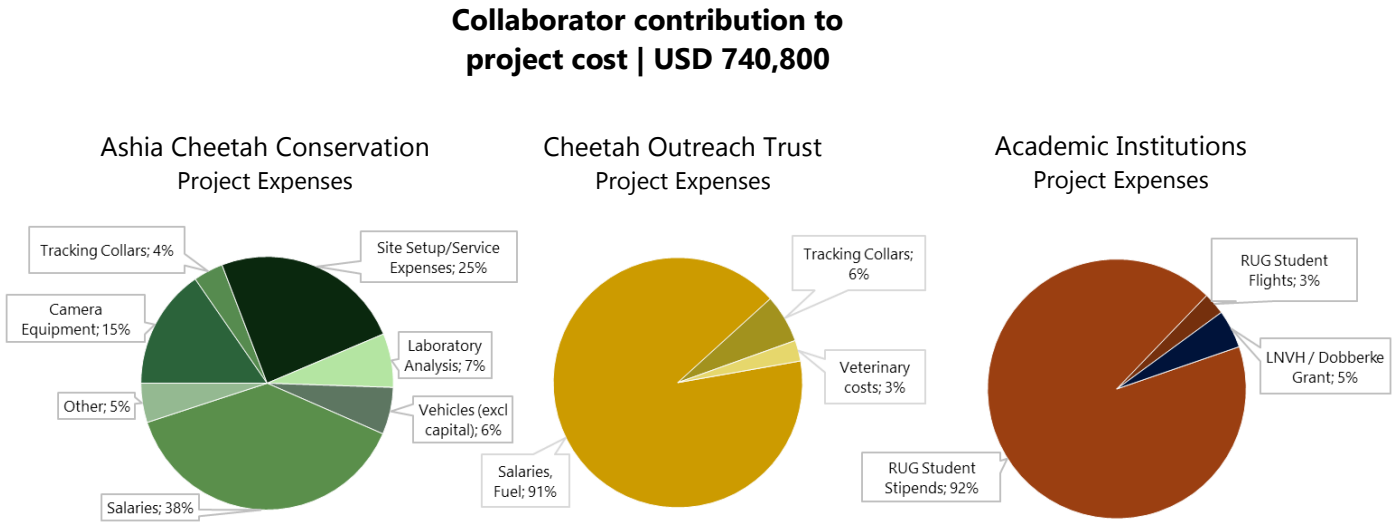

***Fig. 38**: Separate pie charts for the division of expenditures for each collaborator.*

The operational costs (25% of ACC's project expenses) associated with setting up and/or servicing cameras in the field are summarised in the figure below (Fig. 39). Fuel costs were a substantial portion of the total expenditure, highlighting the extensive distances travelled. Over the course of the project, field teams cumulatively drove over 400,000 km, close to the distance from the earth to the moon. Accommodation in isolated areas were limited with only a few suitable options near camera sites. These costs were partially mitigated through discounted rates offered by property owners and, at times, even complimentary accommodation. Where feasible, lower-cost alternatives were used, including camping.

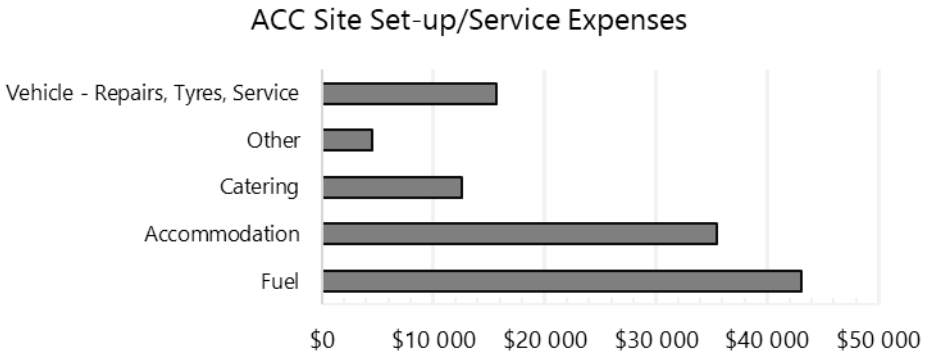

***Fig. 39**: Bar graph showing the break-down of site set-up/service expenses for the entire field work period (Sept 2022 – Dec 2025) for ACC only.*

The substantial human resource investment required to implement the census at this scale was assessed by partitioning full-time equivalent (FTE) contributions into three components: fieldwork, desktop analysis and project oversight. The proportional contribution of each component between 2022 and 2026 is presented in Fig. 40. Fieldwork included all on-site census activities (questionnaire distribution, camera deployment, scat sampling, etc.), while desktop analysis encompassed data processing, image classification, and data management. Project oversight comprised coordination, planning, stakeholder engagement and overall project management.

Field work accounted for the largest proportion of effort overall (60%), reflecting the intensive nature of on-the-ground data collection required for a census of this scale. However, the distribution of effort varied temporally, with earlier phases (2022 – 2023) characterised by higher relative investment in project oversight and setup,

followed by a peak in fieldwork effort during 2024-2025 as data collection intensified. In the final phase (2026), effort shifted toward desktop analysis, reflecting data processing and synthesis as field activities were finalised. In full-time equivalent (FTE) terms, the collective effort of the FRCC equated to ~13 person-years of work (154 months; 4,692 days).

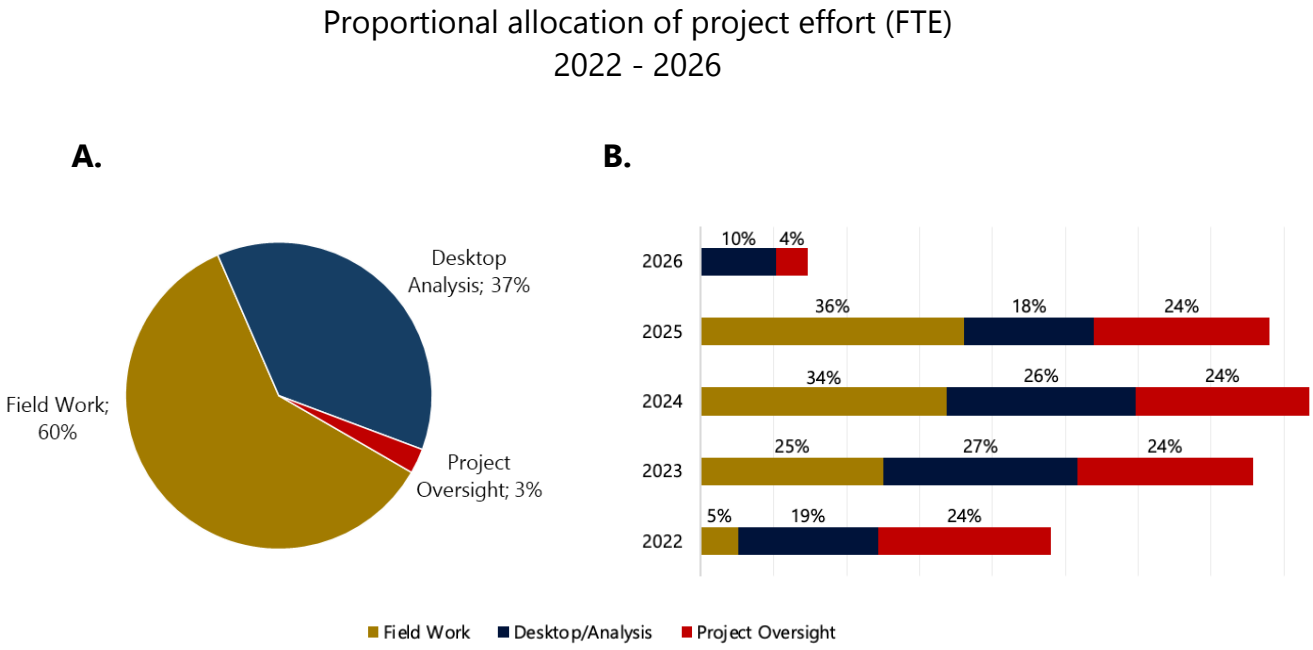

***Fig. 40**: Pie chart for the break-down of project effort through the entire FRCC period. B. A horizontally stacked bar chart to divide the project effort between the three categories across the years for all partners.*

7. Conclusion

The Free-Roaming Cheetah Census was established to address a major knowledge gap in the status and distribution of free-roaming cheetah in South Africa. Previous estimates were largely based on expert opinion, small-scale studies, or extrapolation across extensive areas, which can easily inflate population estimates when the initial data is obtained from cheetah abundant areas. To counter this, the FRCC used a large-scale, direct observational approach based on questionnaire surveys, systematic camera trapping, telemetry, biological sample collection, and DNA analysis (faecal and blood) across the South African IUCN cheetah range (Fig. 41). This provides a more robust basis for a confirmed observed count of cheetah, distribution, and movement than earlier studies that relied heavily on modeled or extrapolated data.

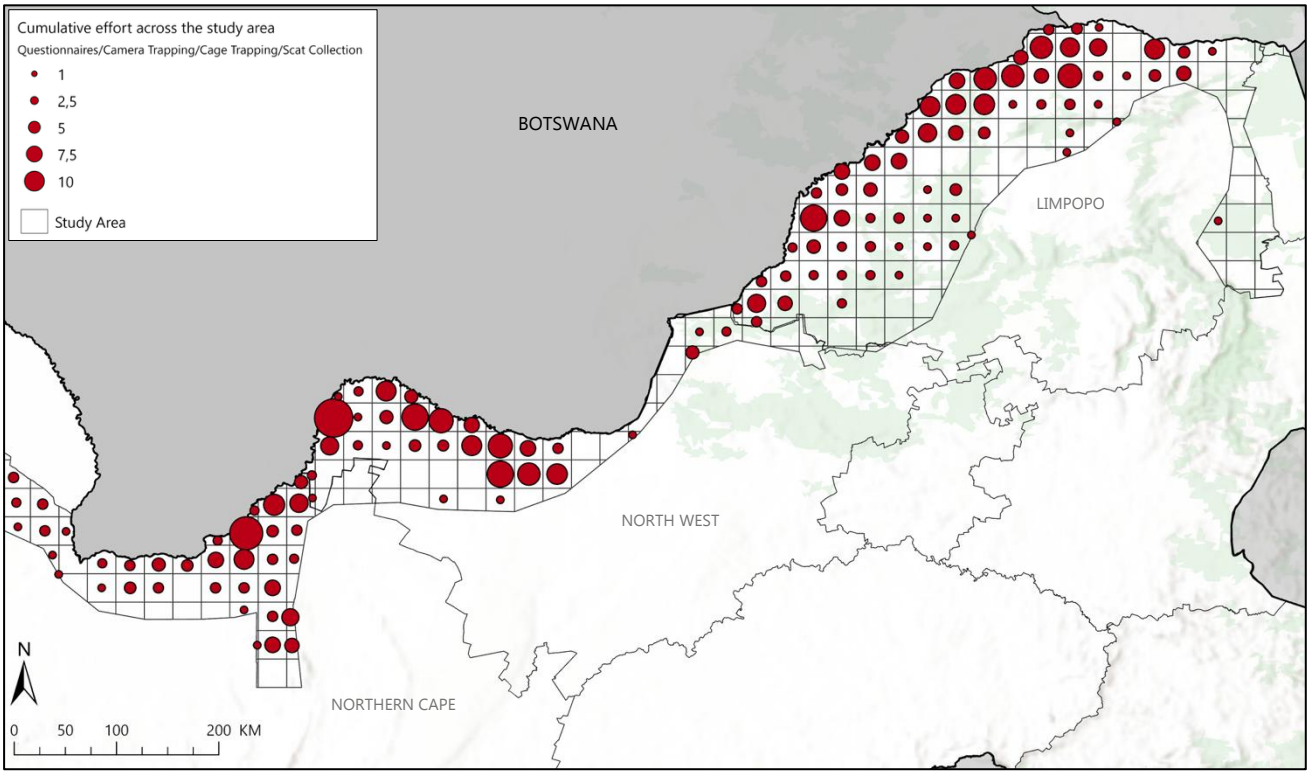

***Fig. 41**: Combined survey effort across the study area in the IUCN cheetah range in South Africa, integrating questionnaire data, camera trapping, cage trapping and scat collection. Sampling intensity is represented by proportional symbol size, with larger circles indicating greater cumulative effort per grid cell.*

The census identified a minimum of 83 adult cheetah individuals across the study area, with an additional 36 young cubs or subadults, totaling to only 119 unique free-roaming individuals. Of the 83 individuals, 68 (82%) had an individual confidence level of four and higher. This individual count is substantially lower than previous estimates.

Weise *et al.* (2017) estimated free-roaming cheetah numbers in southern Africa by applying inferred cheetah densities to broad habitat and ecoregion classifications across areas of confirmed occurrence [97]. If applying this method to the FRCC study area with verified cheetah presence (Fig. 27B), Region 1 (21,875 km^2; semi-arid savanna) should support an estimated population size of ~116 mature individuals, while Region 2 (25,000 km^2; grassland and bushveld), should support ~120 cheetah. However, the FRCC confirmed only a minimum of 33 adult cheetah in Region 1, and 50 adults in Region 2, highlighting the gap between broad-scale density extrapolations, which do not account for demographic characteristics such as age and sex, and documented individuals. Given that the census was conducted over a three-year field period using multiple detection methods, it is unlikely that such a substantial proportion (60 – 70%) of the population remained entirely undetected. This further highlights the uncertainty associated with regional cheetah population estimates,

historically relying on inferred densities, mapped occurrence and extrapolation approaches in the absence of landscape-level empirical surveys [25][79]

Of the adults recorded in the census, 17 individuals had died by the end of the census period, representing 20% of known adult mortality. Such losses are especially concerning in a population that is already very small. The death of this many individuals, particularly reproductively viable adults, slows recovery and makes population growth challenging [46][17]. Adult sex ratios were broadly similar between the two regions; Region 1 showed a female to male ratio of 1:1.8, and Region 2 showed a ratio of 1:1.9, meaning that both regions were effectively close to a 1:2 female to male balance. Because males use sentinel sites more frequently than females, it is likely that female numbers are underrepresented in the data. If female detections matched male detections more closely, adult female numbers may rise slightly, potentially to match the male numbers.

The identified individuals are confirmed to reside in a highly fragmented landscape (Fig. 42) largely caused by differing land use, fencing types, and human population density. Telemetry data confirmed that movement differs between the two main study regions: Region 1 has a more open, arid landscape with extensive connections to Botswana, while Region 2 is more constrained and essentially functions as an isolated population with minor movement between Botswana and PAs in southern Zimbabwe and South Africa, particularly due to dense human settlement and impermeable (game) fencing. Camera-trap data reflected the movement findings

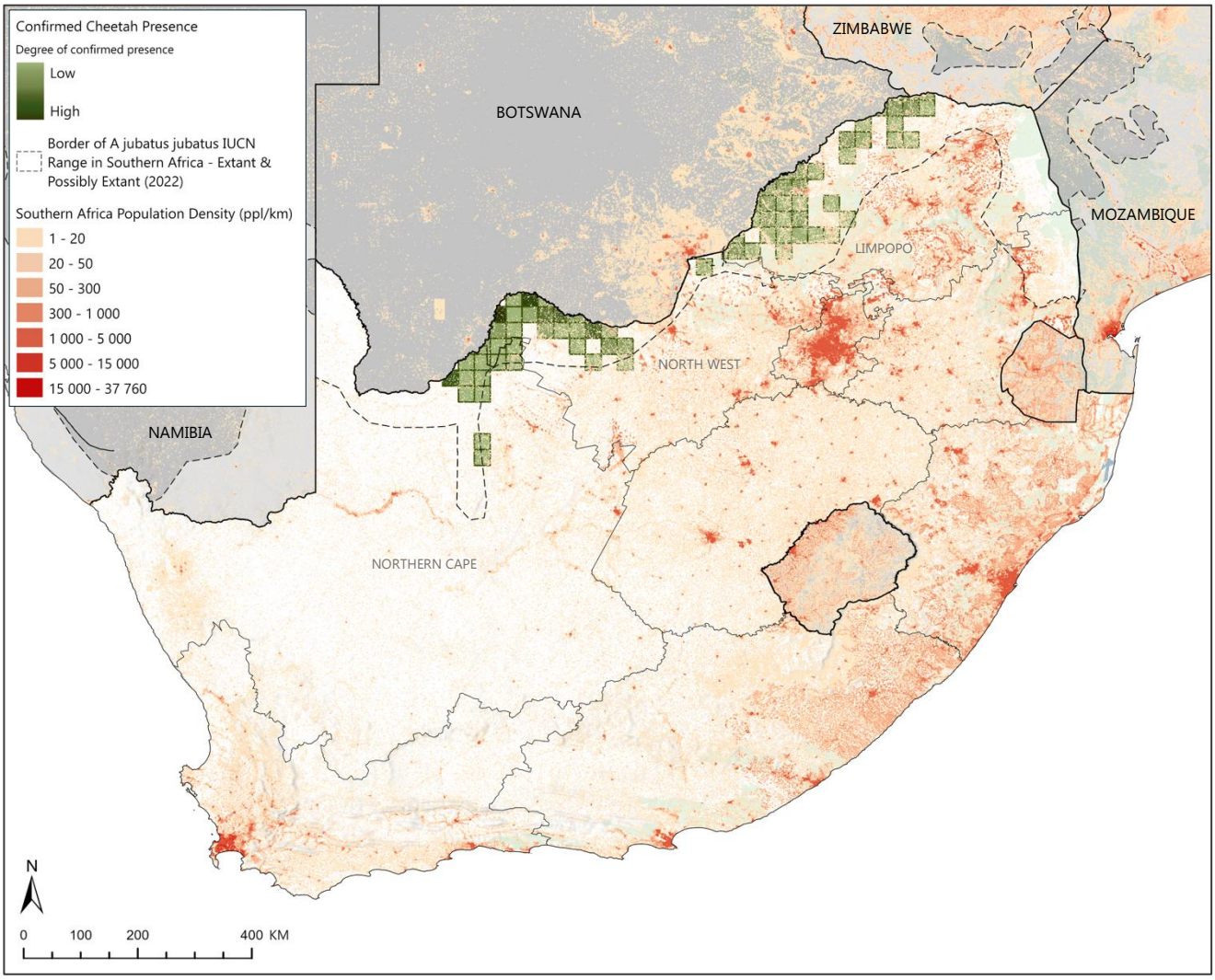

***Fig. 42**: Distribution of confirmed cheetah presence overlaid with human population density across South Africa. Presence is shown as grid cells scaled by degree of confirmation. The overlap between confirmed cheetah occurrence and area of elevated human density underscores the increasing constraints on suitable habitat and the challenges of long-term survival.*

in the cheetahs' activity patterns in Region 2; fragmented land concentrates movement to a small number of areas and therefore increases detection frequency at specific camera sites. This fragmentation prevents connectivity between the regions which in turn increases isolation and potential inbreeding.

However, while land-use and fencing create visible barriers, human perceptions, conflict, and tolerance create boundaries that can be equally destructive for cheetah movement and survival. While many participants associated cheetah with livestock losses, DNA-based scat analysis found prey DNA only from game species, with no evidence of livestock in the cheetah host samples analysed. This indicates that farmer perceptions of cheetah as a livestock threat may overestimate actual predation risk in some areas. Such mismatches between perception and evidence are highly relevant for coexistence planning, as conflict and tolerance are related but not identical (i.e. high conflict does not automatically mean low tolerance).

Questionnaire data also showed that subjective cheetah presence and conflict do not always match objective data from camera traps, GPS collars, and found cheetah scat. In some areas, cheetah were reported where no objective evidence was found, suggesting that tolerance, prior experiences, and perceptions can strongly shape how people report presence. Adding these subjective types of information to range maps that use habitat and population density data is important as areas with low tolerance may become functionally unsuitable even when habitat appears suitable. At present, the legal and administrative process following the hunting and killing of a cheetah is difficult to interpret and even harder to enforce in practice. Although cheetah are legally protected, the procedure that goes from incident to meaningful legal action is unclear. To date, no known cheetah killing has resulted in a successful prosecution, which highlights a significant gap between legislation and implementation.

Despite the physical and social fragmentation of the landscape, the genetic results are still indicative of a relatively connected population. At this stage, biological fragmentation between the two regions does not appear to be strong yet, suggesting that gene flow has occurred relatively recently and that the two regions have remained genetically connected up to now. However, as aforementioned, the ecological evidence does point to increasing restriction of movement between the regions, particularly where human population density and impermeable fencing create barriers. Genetic differentiation in long-lived mammal populations, like the cheetah, often becomes detectable only after several generations, typically in the order of 5–15 generations. Given a cheetah generation time of ~4–6 years, this suggests that measurable genetic structuring may lag behind landscape fragmentation by several decades [2][33]. Thus, although full genetic separation has not yet occurred, the conditions are in place for this process to continue if connectivity declines further. The likely isolation of the population in Region 2 (N_{Adults} = 50) due to movement being restricted by human settlement are especially vulnerable to genetic drift, inbreeding, and the gradual loss of genetic diversity, already indicated by the positive inbreeding coefficient (F_{IS} = 0.155). With a minimum effective population of 100 needed to limit short-term inbreeding effects [68], the current Region 2 population size suggest that it may already be approaching levels associated with future genetic erosion. This provides an important conservation opportunity: if connectivity can be maintained, genetic exchange may support population resilience and reduce long-term isolation ris

The FRCC findings have direct relevance for national and international policy change. The substantially lower population estimate identified reflects a combination of improved survey accuracy resulting from the first coordinated cross-country census, as well as ongoing population declines driven by habitat loss, landscape fragmentation and anthropogenic pressures. The Regional Conservation Strategy for Cheetah and Wild Dog in Southern Africa (2015) adjusted the population estimate for free-roaming cheetah in unprotected range in South Africa to ~300 individuals [72]. This new count contributed to the population data utilized by the IUCN Red List Assessment (2021), which estimated 3526 adult individuals in Southern Africa, retaining its Vulnerable status [26]. In contrast, the FRCC detected only 83 adult individuals, representing an approximate 72% lower estimate than previously assumed. These findings point towards the potential reclassification of the national free-roaming population to Endangered status under the Regional Red List of South Africa criteria, despite the species not necessarily qualifying as Endangered globally just yet. The study also supports updating the IUCN range for

southern African cheetah in South Africa. The new range should be based on a combination of confirmed presence, potential suitable habitat, land-use, fragmentation, and social factors affecting the persistence of cheetah in the landscape.

The FRCC data are intended to inform the ongoing South African Biodiversity Management Plan for cheetah and should be used to strengthen future monitoring and reporting. Given the scale and complexity of the landscape, continued census work will be necessary, ideally at regular intervals using standardised protocols that allow comparisons through time. However, since projects of this scale require sufficient time, funding and people, appropriate planning and teamwork are of the utmost importance.

The report also highlights the need for coordinated action across the different cheetah populations in South Africa, including free-roaming cheetah, the semi-protected populations in Kruger NP and Kgalagadi TP, the metapopulation in fenced reserves and the captive population. A more connected national, and eventually southern African regional approach will be essential if South Africa is to maintain viable free-roaming cheetah populations into the future.

8. Case studies

The following case studies are included to highlight important findings from the FRCC. Together, they illustrate the biological, social, and legal dimensions of free-roaming cheetah conservation in South Africa. Some cases demonstrate population resilience and rare biological events, while others reveal the continuing challenges of conflict, coexistence, and law enforcement. These case studies provide a more detailed view of relationships between cheetah and people in the study area.

8.2 Persistence under pressure: cub survival in a challenging landscape

In July 2024, an adult female cheetah was first recorded in grid NW_G6 in Region 1, accompanied by a litter of remarkable size, consisting of seven cubs (Fig. 43). Based on their size and development, the cubs were estimated to be 6-7 months of age at the first observation. This is notable as cheetah litter sizes are typically much smaller, with an average of three cubs [86][5]. Although larger litters have been documented, for example, a litter of eight was recorded in captivity in 2017, and more recently, another litter of seven was observed in Kruger NP, these instances are rare. Cub mortality is usually very high, with less than half of the cubs surviving into sub-adulthood [86]. The fact that seven cubs had already survived to this stage therefore represented an unusual and encouraging reproductive event for the free-roaming population.

***Fig. 43**: Individual FRCC_068 seen with her seven cubs in grid NW_G6.*

The F and G grids in North West, Region 1, were characterised by high levels of perceived conflict with cheetah. The grid in which the mother and cubs were first recorded also showed exceptionally low tolerance towards predators in general (Fig. 30), suggesting a challenging social landscape for cheetah persistence in the area. When first observed, the mother and cubs remained at a sentinel tree for approximately 20 minutes and appeared relaxed.

The family group was later encountered again for a time span of 13 minutes on 31 May 2025 in a nearby grid, NW_F5, where only four of the original seven cubs, now sub-adults, were found without the mother. At this

stage, the sub-adults were approximately 15 months old, which is toward the lower end of the age range at which young cheetah may begin to become independent, typically between 15 and 20 months [48]. Three of the four individuals were females, and female individuals are not expected to form coalitions in the same way as males. This suggests that the four were likely still associated as a sibling group rather than functioning as independent adults just yet. The mother may have dispersed to a new area for mating opportunities or may have been killed.

The absence of the remaining three sub-adults most likely indicates that they did not survive. Notably, the F5 grid in which the sub-adults were found again, despite also showing high conflict, was characterised by high tolerance towards predators (Fig. 30). This contrasts with the G6 grid where the mother and litter were first observed. It is possible that the sub-adults later remained in this more tolerant grid after supposed familial losses.

A few months later, on 19 August 2025, only two of the sub-adults were encountered again, both females. Their continued association is consistent with an immature sibling group, and the disappearance of the other two individuals from the group suggests additional mortality from unknown causes. Even with only two individuals confirmed to be alive, and five of the seven individuals likely deceased, the case remains remarkable. It demonstrates that, under the right conditions, cheetah in the free-roaming landscape can successfully raise exceptionally large litters to near sub-adulthood, despite the many challenges facing cub survival in fragmented farmland systems.

8.3 Site loyalty in the Northern Cape

In August 2024, a coalition of two male cheetah was first recorded at a sentinel site in grid NC_W8 in Northern Cape, Region 1 (Fig. 44). From that point onward until their last confirmed detection in November 2025, the coalition returned to the same sentinel site with remarkable frequency. On average this coalition revisited the site every two days (Fig. 24A), and on some occasions multiple times within a single day, resulting in a total of 3,573 images of these cats. They were also observed at a second site, on average every 11 days, on the same farm. This repeated and consistent use of the same area suggests a high level of site loyalty and local familiarity, even though the landscape of Region 1 is relatively open and permeable.

The farm they occupy covers approximately 4,000 ha (40 km²) and is fenced only with small (2-4-strand) fencing, making it relatively large and easily traversable. In other words, it is unlikely that these males are "trapped". Yet despite the lack of strong fencing barriers, and the absence of potential mates in the surrounding area (no other individuals have been found in the Northern Cape province), the coalition continued to return.

Fig. 44*: Male coalition (FRCC_062 and FRCC_063) in the NC_W8 grid. Male 063 is seen with blood on his face, with male 062 walking in the back.*

Their behaviour is also interesting in the context of previous work from central Namibia, where the Leibniz Institute for Zoo and Wildlife Research has shown that male cheetah follow different spatial tactics The more static males, similar to this coalition, maintain smaller territories of around 380 km², while others can have home ranges up to 1,600 km² [54]. The two males recorded in Region 1 may have been displaying a territorial strategy, but their repeated use of a single area and the lack of other cheetah in the surrounding area makes this behaviour difficult to classify within the typical patterns described elsewhere [21]. It is possible that they are older males, already having mated, that have settled into a low-conflict, relatively tolerant area with sufficient prey and safe access. Regardless, their use of the landscape stays unusual compared to what would normally be expected for a coalition.

A total of 22 scat samples were collected in the area, all attributable to these two males specifically, marking the area as an important communication hub. While the owner of this specific farm, together with the landowners

of the surrounding farms (N = 4), report that the area is largely used for livestock farming and only some game farming, DNA analysis found **no livestock in their diet**. Instead, the samples contained mostly small game species such as springbuck and steenbok. This is consistent with the camera-trap records within this grid, which also showed frequent marking and defecation events, as well as footage of the males with bloodied faces (Fig. 44), suggesting hunting and repeated use of the area as part of a core movement route or hunting ground.

The farm owner indicated that while predators were not preferred, cheetah were tolerated if they simply passed through the area rather than causing damage. Other questionnaire data, including the landowners in the surrounding area, show that overall tolerance for cheetah was high and no cheetah conflict was reported (Fig. 30). This is important as it indicates that the social environment may have contributed to the coalition's continued use of the area.

Since the landowners in this area have reported no loss of livelihood, and faecal analysis has proven that no livestock has been depredated, there appears to be a form of peaceful coexistence between people and cheetah in this area, where the species is tolerated rather than persecuted. This case study exemplifies the kind of human-wildlife coexistence that needs to be fostered through conservation efforts, where tolerance and minimal conflict can safeguard cheetah populations for the future.

8.4 Persecution without prosecution: tolerance and enforcement gaps in a human-dominated landscape.

In 2021, a year before the start of the census fieldwork, COT obtained camera trap images of a coalition of two male cheetah in grid LP_I4, in Limpopo, Region 2. It was later reported that one of the coalition mates had been shot by a local farmer, though the farmer remained unidentified at the time. During the first year of our census, in September 2022, the surviving male reappeared as our first identified individual in the same grid, earning the name FRCC_001. This individual exhibited strong site loyalty within LP_I4, moving between three sentinel sites on two different farms. He showed a clear preference for one site, revisiting it on average every four days (Fig. 24B), compared to much less frequent revisits to the other two (with averages of 94 days and 143 days), resulting in being captured on camera 1,643 times.

On the 21st of February in 2024, this lone male was fitted with a satellite collar and monitored for a total of 137 days until his death on the 7th of July 2024. The collar data helped with the determination of the cause of death, concluding that this male had also been shot, like his coalition mate, by a local farmer. As outlined in the policy chapter (section 5), cheetah are protected under South Africa's Threatened or Protected Species Regulations due to their IUCN Vulnerable status and CITES Appendix I listing. Killing or harming a cheetah without a permit constitutes a serious offence, punishable by fines up to three times the animal's commercial value and imprisonment for up to five years. Yet, prosecuting such cases remains exceedingly challenging, most likely due to low priority given to conservation cases. In this instance, the farmer openly admitted to the shooting, but no penalties were enforced due to a lack of evidence for "ill intent". When even an admission of killing a protected species fails to trigger prosecution, conservationists are left powerless, and legal protections that are intended to safeguard the species do not provide protection at all.

Following this incident, COT adopted a more proactive approach upon learning of another cheetah mortality event. A coalition of four males had been frequently detected on camera traps across grids LP_E7 and LP_F7 in Limpopo, Region 2, spanning three farms. One male from this coalition was collared on the 27th of August in 2024 and recollared on the 3rd of June in 2025, only to die three weeks later. Collar data again pinpointed the cause, confirming that the individual had been hit by a vehicle, as had two of his coalition mates, all three killed in the incident. A local informant contacted COT, reporting that the act appeared deliberate, with the vehicle intentionally aiming for the cheetah (Fig. 45). Due to the previous negative experience of following formal legal channels, COT offered a R100,000 (~6,100 USD) reward instead, funded by private donors, for information identifying the perpetrator. However, this strategy backfired, eliciting complaints from farmers who questioned why substantial funds existed for cheetah investigations but not for their own livelihood losses due to depredation.

Fig. 45*: One of the males, FRCC_050, from the four male coalition. This image was sent to COT by an informant after the vehicle collision.*

These cases expose an important issue in human-cheetah conflict resolution. Prosecution fails to deter offenders, while reward schemes for mortality investigations foster resentment among farmers. These dynamics decrease tolerance towards both predators and conservationists. The path forward demands robust, enforceable rules paired with governmental support, which does not have to directly involve financial compensation, but could also provide conflict preventing interventions (i.e. subsidized guard dogs or partially financed fencing) to foster a higher tolerance towards predatory species like the cheetah.

8.5 Rare king cheetah sighting in the study landscape

A rare phenotypic variant (i.e. physical characteristic) mutation in cheetah is known as the king or tabby phenotype which results in a unique coat pattern of blotchy connected spots and dark stripes that run across its back. Once two individuals with the mutated gene have been identified and paired, their offspring has a one-in-four (25%) chance of being born a king. It can however also skip generations. The genes that cause this mutation are recessive genes, meaning that they only come to expression when the individual inherits the recessive allele from both parents. King cheetah have been recorded sporadically over the past 100 years [40] in southern Africa and was unfortunately first identified by the western world by being shot at. Initially the mutation was thought to be a hybrid of leopard and cheetah, or hyaena and cheetah, by mistaken locals. Later the mutation was wrongly assigned as a new species, *Acinonyx rex*, until it was eventually corrected in 1939 when the blotched pattern was ascribed to genetic variation.

Considering this, we were unprepared to see a king cheetah on camera, which to our knowledge, is the first credible sighting since 1986 in Kruger NP and according to unpublished reports, again in the mid-2000's. (Fig. 46; Appendix VI). This implies important genetic implications for the broader population as its expression indicates that this rare allele is still present and being successfully transmitted.

***Fig. 46**: King cheetah individual captured on camera-trap during the census in 2023. Location will not be disclosed.*

However, recessive traits may become more frequently expressed in small or partially inbred populations as inbreeding increases homozygosity (carrying two identical alleles of a gene), allowing alleles that are normally masked in heterozygous individuals to be expressed visually [10]. This finding reinforces the fact that maintaining numbers alone is not enough to conserve the last free-roaming cheetah in South Africa, but that the underlying genetic diversity that enables populations to persist and adapt over time must be safeguarded.

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- Marna Smit — Director, Ashia Cheetah Conservation

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- Marna Smit — Project management, field coordination, report writing and scientific analysis
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Appendices

Appendix I – Free-Roamer Cheetah Census Questionnaire
Appendix II - Predator Recognition Kit
Appendix III – Camera Trapping and Scat Collection Procedures
Appendix IV – Site Operations
Appendix V – Camera Trap Report
Appendix VI – FRCC ID Kit
Appendix VII – Unistel Genetics Report

Free-Roamer Cheetah Census

Welcome. This survey is a collaborative effort between the Cheetah Outreach Trust, Ashia Cheetah Conservation, Stellenbosch University and the University of Groningen.

What type of survey will you be completing?

Please provide if you will do the survey by yourself, face-to-face or over the phone

- ○ No interviewer present
- ○ Face-to-face
- ○ Telephone

0. Starting information

0.1. Interviewer

Please provide who takes the interview

- ○ Deon Cilliers
- ○ Other

0.1.1. Who?

"Other" was selected. Please provide who takes the interview

0.2. Current location

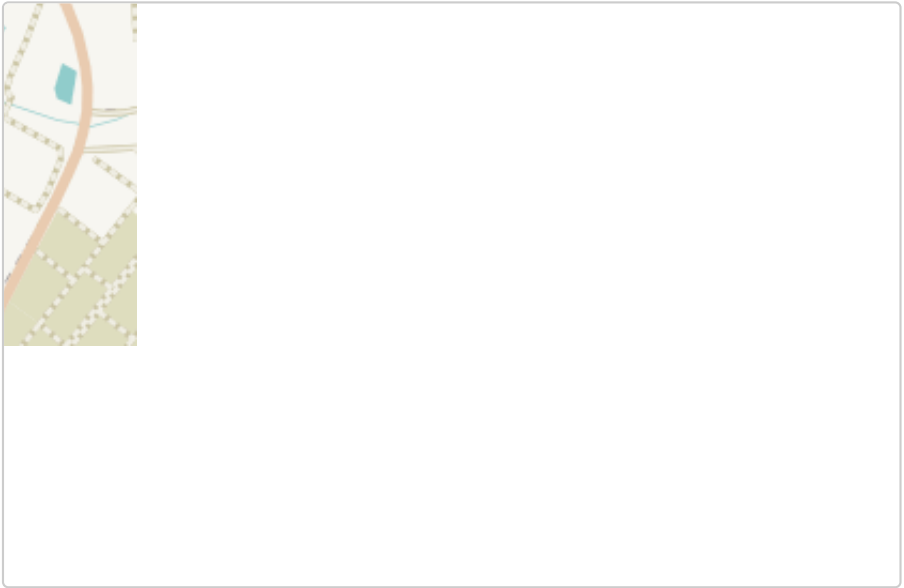
Please add the current location of the interviewer

latitude (x.y °)

longitude (x.y °)

altitude (m)

accuracy (m)



Do you want to stay anonymous throughout the survey?

Please provide if you mind giving us personal details (e.g. name, exact address and phone number)?

- ○ Yes
- ○ No

1. Farmland and owner information

1.1. Name and Surname

Please provide your name and surname

_____

1.2. Role on farmland

Please provide your function at the farm

- ○ Owner
- ○ Manager
- ○ Laborer
- ○ Other

1.2.1. What role?

You selected "Other". Please provide what function you have on the land

_____

1.3. Residential address (including nearest town or city)

Please provide a full permanent residential address for the farm owner or manager (whomever is completing the survey), which includes a property number or street address and the nearest town or city

_____

1.4. Contact number

Please provide a phone number on which you can be contacted

_____

1.5. Email address

Please provide your email adress

_____

1.6. Country

Please select the country in which the farm is located

- ○ South Africa
- ○ Zimbabwe
- ○ Botswana
- ○ Other

1.6.1. What country? (Other)

You selected "Other". Please provide in what country the farm is located

---

1.6.1. Province (SA)

Please select the province in which the farm is located

- ○ Limpopo
- ○ Northern Cape
- ○ North West
- ○ Other

1.6.1.1. What Province? (Other)

You selected "Other". Please provide at what province the farm is located

---

1.7. Click here to go to a map of Limpopo

Please be patient for the grid layer to load in. Zoom in or click on the grid that overlaps with your farm to see the label, which can be entered in the next question.

1.7.1. Enter the grid letter and number

Please enter the letter and number of the grid cell in LP where the farm is located in seen in the previous question

---

1.7. Click here to go to a map of Northern Cape

Please be patient for the grid layer to load in. Zoom in or click on the grid that overlaps with your farm to see the label, which can be entered in the next question.

1.7.1. Enter the grid letter and number

Please enter the letter and number of the grid cell in NC where the farm is located in seen in the previous question

---

1.7. Click here to go to a map of North West

Please be patient for the grid layer to load in. Zoom in or click on the grid that overlaps with your farm to see the label, which can be entered in the next question.

1.7.1. Enter the grid letter and number

Please enter the letter and number of the grid cell in NW where the farm is located in seen in the previous question

---

1.7. Estimated farm location

Please provide the location of your farm if the gps is working. Otherwise, skip this question

latitude (x.y °)

longitude (x.y °)

altitude (m)

accuracy (m)

1.8. Farm name(s) and property number(s)

Please provide the name and property number of your farm

1.9. How long have you been on the farm?

Please provide an estimation of how long ago you first started farming on the property

- 1-5 years
- 6-10 years
- >10 years

1.10 What is the total size of the farm?

Please provide an estimated size of the farm(s) in ha or km2 (and specify which)

1.11 How much of the farm is fenced and what type is used?

Please provide what type of fencing you use around your farm. Multiple answers possible

	None	Some	Mostly	Only
Small (2-4) strands				
Large (game-proof)				
Large (predator-proof)				
No fence				

Other	○	○	○	○

1.11.1. What fence type?

You selected "Other". Please provide what fence type you use around the farm(s)

---

2. Land use

2. Land use *Please estimate what proportion of each type of farming you practice*	None	Some	Mostly	Only
2.1. Agriculture	○	○	○	○
2.2. Ecotourism	○	○	○	○
2.3. Game	○	○	○	○
2.4. Livestock	○	○	○	○
2.5. Other	○	○	○	○

» 2.1. Agriculture

2.1.1. What do you grow?

You selected "Agriculture". Please provide information

2.1.2. Pests on property

Please provide if you have pests on your property

- ○ Yes
- ○ No

2.1.2.1. What pests?

You selected "Yes". Please provide details as to what pests are on your land

---

2.1.2.2. How do you deal with these pests

Please provide how you deal with pests on your property. Multiple answers possible

- ☐ Pesticides (sprays etc)
- ☐ Plants
- ☐ Shoot
- ☐ Traps
- ☐ Other

2.1.2.2.1. What other control methods?

You selected "Other". Please provide details of what you use

» 2.2. Ecotourism

2.2.1. How often do tourists visit your farm annually?

You selected "Ecotourism". Please provide information

- ○ Daily
- ○ Weekly
- ○ Monthly
- ○ Seasonally

2.2.2. Do you provide conservation benefits?

Please provide if you supply any conservational benefits

- ○ Yes
- ○ No

2.2.2.1. What benefits?

You selected "Yes". What benefits do you provide?

2.2.3. Do you educate tourists?

Please provide if you educate the tourists that visit your land

- ○ Yes
- ○ No

2.2.3.1. How?

You selected "Yes". Please provide how you educate tourists

» 2.3. Game farming

2.3.1. Which form of game farming is done on your property?

You selected "Game". Please provide information. Multiple answers possible

- [ ] Free range game
- [ ] Game sales
- [ ] Hunting
- [ ] Intensive breeding
- [ ] Personal use
- [ ] Stock farming
- [ ] Other

2.3.1.1. What range? (Hunting)

You selected "Hunting". Please provide if you hunt locally or internationally

- ( ) Local hunting
- ( ) International hunting

2.3.1.1. Which form? (Other)

You selected "Other". Please provide which form of game farming you execute

---

2.3.2. Do you do formal game counts?

Please provide if you count your game formally

- ( ) Yes
- ( ) No

2.3.2.1. What methods do you use to count/monitor your game?

Please provide which methods you use to do formal game counts. Multiple answers possible

- [ ] Helicopters
- [ ] Drinking pint surveys
- [ ] Physical counting
- [ ] Local knowledge
- [ ] Unique ID
- [ ] Other

2.3.2.1.1. What methods? (Other)

You selected "Other". Please provide what other method(s) you use to count your game

---

2.3.2.1.1. How are your game identified? (ID)

You selected "Unique ID". Please provide how you identify your game

- [ ] Collars
- [ ] Ear notch
- [ ] Ear tags
- [ ] Other

2.3.2.1.1.1. How? (Other)

You selected "Other". Please provide how you identify your game

---

2.3.2.2. When was your last game count?

Please provide when you last counted your game

2.3.3. Game numbers *Which game/wildlife species do you have and how often is each species seen. Select species that form part of your game*	Never	Rarely	Often	Uncertain
Aardvark	○	○	○	○
Blesbuck	○	○	○	○
Black back jackal	○	○	○	○
Black wildebeest	○	○	○	○
Blue wildebeest	○	○	○	○
Bontebok	○	○	○	○
Brown hyena	○	○	○	○
Buffalo	○	○	○	○
Burchell zebra	○	○	○	○
Bushbuck	○	○	○	○
Bushpig	○	○	○	○
Caracal	○	○	○	○
Cheetah	○	○	○	○
Crocodile	○	○	○	○
Duiker	○	○	○	○
Eland	○	○	○	○

Elephant	○	○	○	○
Gemsbok	○	○	○	○
Giraffe	○	○	○	○
Impala	○	○	○	○
Klipspringer	○	○	○	○
Kudu	○	○	○	○
Leopard	○	○	○	○
Lion	○	○	○	○
Mountain reedbuck	○	○	○	○
Nyala	○	○	○	○
Ostrich	○	○	○	○
Red hartebeest	○	○	○	○
Roan	○	○	○	○
Sable	○	○	○	○
Serval	○	○	○	○
Spotted hyena	○	○	○	○
Springbok	○	○	○	○
Steenbuck	○	○	○	○
Tsessebe	○	○	○	○
Warthog	○	○	○	○
Waterbuck	○	○	○	○
Wild dog	○	○	○	○
Other	○	○	○	○

2.3.3.1. What game?

You selected "Other". Please provide details on what game

2.3.4. Do you suffer annual game losses?

Please provide if you lose any of your game each year

- Yes
- No

2.3.4.1. Estimated annual game losses

You selected "Yes", Please provide the annual game losses you have per species

	None	Daily	Weekly	Monthly	Seasonally
Aardvark					
Blesbuck					
Black backed jackal					
Black wildebeest					
Blue wildebeest					
Bontebok					
Brown hyena					
Buffalo					
Burchell zebra					
Bushbuck					
Bushpig					
Caracal					
Cheetah					
Crocodile					
Duiker					
Eland					
Elephant					
Gemsbok					
Giraffe					
Impala					
Klipspringer					
Kudu					
Leopard					

Lion	○	○	○	○	○
Mountain reedbuck	○	○	○	○	○
Nyala	○	○	○	○	○
Ostrich	○	○	○	○	○
Red hartebeest	○	○	○	○	○
Roan	○	○	○	○	○
Sable	○	○	○	○	○
Serval	○	○	○	○	○
Spotted hyena	○	○	○	○	○
Springbok	○	○	○	○	○
Steenbuck	○	○	○	○	○
Tsessebe	○	○	○	○	○
Warthog	○	○	○	○	○
Waterbuck	○	○	○	○	○
Wild dog	○	○	○	○	○
Other	○	○	○	○	○

2.3.4.2. Reason for losses

Please provide what could have caused game losses. Multiple answers possible

- ☐ Disease
- ☐ Extreme weather effects (drought)
- ☐ Natural cause
- ☐ Predation events
- ☐ Theft
- ☐ Other

2.3.4.2.1. What reasons?

You selected "Other". Please provide what caused your game losses

---

» 2.4. Livestock

2.4.1. When was your last livestock count?

You selected "Livestock". Please provide information

2.4.2. What livestock do you have? *Please provide what livestock you own*	None	Some	Mostly	Only
Cattle	○	○	○	○
Goats	○	○	○	○
Ostrich	○	○	○	○
Sheep	○	○	○	○
Other	○	○	○	○

2.4.2.1. What livestock?

You selected "Other". Please provide what livestock you own

2.4.3. Do you have a calving, lambing and/or laying season?

Please provide if you have a calving, lambing and/or laying season

- ○ Yes
- ○ No

2.4.3.1. When is your calving, lambing and/or laying season?

You selected "Yes". Please provide us with the correct season

- ☐ Spring
- ☐ Summer
- ☐ Autumn
- ☐ Winter
- ☐ Year round
- ☐ Other

2.4.3.1.1. When?

You selected "Other". Please provide when your calving season is

---

2.4.4. Do you suffer annual livestock losses?

Please provide if you lose any of your livestock each year

- ○ Yes
- ○ No

2.4.4.1. Estimated annual livestock losses

You selected "Yes". Please provide an estimate of how much livestock you lose in a year

	None	Daily	Weekly	Monthly	Seasonally
Cattle	○	○	○	○	○
Goats	○	○	○	○	○
Ostrich	○	○	○	○	○
Sheep	○	○	○	○	○
Other	○	○	○	○	○

2.4.4.2. Reason for losses

Please provide what could have caused livestock losses. Multiple answers possible

- ☐ Disease
- ☐ Extreme weather effects (drought)
- ☐ Natural cause
- ☐ Predation events
- ☐ Theft
- ☐ Other

2.4.4.2.1. What reason?

You selected "Other". Please provide what caused your livestock losses

2.4.4.3. Estimated annual financial losses

Please provide an estimate of how much rand you lose in a year

2.5. What type of farming do you practise on your land?

You selected "Other" as one of your farming types. Please provide what you practise

3. Free-roaming wildlife

3.1. Do you have free-roaming wildlife on your property?

Please provide if any free-roaming wildlife is seen on your land

- ○ Yes
- ○ No

3.1.1. Wildlife numbers

You selected "Yes". Which wildlife species do you have and how often is each species seen

	Never	Rarely	Often	Uncertain
Aardvark	○	○	○	○
Blesbuck	○	○	○	○
Black wildebeest	○	○	○	○
Blue wildebeest	○	○	○	○
Bontebok	○	○	○	○
Buffalo	○	○	○	○
Burchell zebra	○	○	○	○
Bushbuck	○	○	○	○
Bushpig	○	○	○	○
Crocodile	○	○	○	○
Duiker	○	○	○	○
Eland	○	○	○	○
Elephant	○	○	○	○
Gemsbok	○	○	○	○
Giraffe	○	○	○	○
Impala	○	○	○	○
Klipspringer	○	○	○	○
Kudu	○	○	○	○
Mountain reedbuck	○	○	○	○
Nyala	○	○	○	○
Ostrich	○	○	○	○
Red hartebeest	○	○	○	○
Roan	○	○	○	○
Sable	○	○	○	○
Springbok	○	○	○	○
Steenbuck	○	○	○	○

Tsessebe	○	○	○	○
Warthog	○	○	○	○
Waterbuck	○	○	○	○
Other	○	○	○	○

3.1.1.1. What wildlife?

You selected "Other". Please provide what wildlife species occur on your land

---

4. Predator occurrences

4.1. Do free-roaming predators occur on your land?

Please provide if any free-roaming predators are seen on your land

- ○ Yes
- ○ No

4.1.1. Which predators and how often? *You selected "Yes". Please provide details on which predators and how often you see them*	Never	Rarely	Often	Uncertain
African wildcat	○	○	○	○
Black backed jackal	○	○	○	○
Brown hyena	○	○	○	○
Caracal	○	○	○	○
Cheetah	○	○	○	○
Leopard	○	○	○	○
Lion	○	○	○	○
Serval	○	○	○	○
Spotted hyena	○	○	○	○
Wild dog	○	○	○	○
Other	○	○	○	○

4.1.1.1. Which predators? (Other)

You selected "Other". Please provide which predators and how often you see them on your land

---

4.1.1.1. How often do you find cheetah signs on your land? (Cheetah)

You selected "Cheetah". Please provide how often you find cheetah signs on your land

	Never	Rarely	Often	Uncertain
Spoor	○	○	○	○
Feces	○	○	○	○
Physical sightings	○	○	○	○

4.1.1.2. Do you have cheetah marking spots on your farm?

You selected "Cheetah". Please provide if you have cheetah marking spots

- ○ Yes
- ○ No
- ○ Uncertain

5. Conflict

5.1. Are you often in conflict with any of these predators?

Please provide if you find any problems with these predators on your land

	Yes	No
African Wildcat	○	○
Black backed jackal	○	○
Brown hyena	○	○
Caracal	○	○
Cheetah	○	○
Leopard	○	○
Lion	○	○
Serval	○	○
Spotted hyena	○	○
Wild dog	○	○
Other	○	○

5.1.1. What predators? (Other)

You selected "Other". Please provide with what predators you are in conflict

_____

5.1.1. How often do you experience unwanted interactions annually

You selected "Yes". Please provide an estimate of how often these unwanted interactions take place per year

- Daily
- Weekly
- Monthly
- Seasonally

5.2. What is your attitude towards predators? *Please provide how you feel about these statements on predator interactions*	Strongly disagree	Disagree	Neutral	Agree	Strongly agree	No comment
I am okay with large predators on my land						
I do not mind predators passing though my land						
Small predators do not aggrevate me						
I do not tolerate large predators						

» 5.3 Predator recognition

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.1. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

5.3.2. What predator is this?

---

6. Predation management

6.1 What type of interventions do you use/have you used against predators?

Please mark what you use or have used in the past. Multiple answers possible

- [ ] I do not use interventions
- [ ] Conflict prevention
- [ ] Guard dogs
- [ ] Removal of individuals
- [ ] Retaliation (after conflict has already appeared)
- [ ] Other

6.1.1. What interventions? (Other)

You selected "Other". Provide what interventions you use

_____

6.1.1 Relocation to nature reserves? (Removal)

You selected "Removal of individuals". Please provide if you also removed some/all of these individuals to nature reserves

- Yes
- No

6.1.1. The interventions helped *Please provide if any of your used interventions have helped*	Strongly disagree	Disagree	Neutral	Agree	Strongly agree	No comment
Conflict preventing						
Guard dogs						
Removal (live capture and remove)						
Retaliation (lethal)						
Other						

6.1.2. Are only the targeted species affected by the interventions?

Please provide if only the targeted species were affected or also any additional species

- Yes
- No

6.1.3. Estimated annual intervention costs

Please provide an estimation of how much rand your interventions cost yearly

_____

6.2. Do you get governmental support for any losses/damage done by predators?

Please provide if the government supplies assistance for any losses/damage done by predators

- Yes
- No

7. Predator removal and relocation

7.1. Have you ever removed predators of your land?

Please provide if you have removed predators of your land in any shape or form

- Yes
- No

7.1.1. Who can assist you with removals?

You selected "Yes". Please provide who assisted you with the removals (e.g. NGO's, other farmowners, private)

7.1.2. Which species?

You selected "Yes". Please provide which species you have removed from your land

	Yes	No
African wildcat	○	○
Black backed jackal	○	○
Caracal	○	○
Cheetah	○	○
Hyena	○	○
Leopard	○	○
Lion	○	○
Serval	○	○
Other	○	○

7.1.2.1. Which other species? (Other)

You selected "Other". Please provide which other species have been removed from your land

---

7.1.2.1. Which methods did you use to remove cheetah? (Cheetah)

You selected "Cheetah". Please mark the methods applied. Multiple answers possible

- ☐ Cage trapping
- ☐ Free darting
- ☐ Gin trapping
- ☐ Relocation to other areas (e.g. by car or helicopter)
- ☐ Shooting
- ☐ Other

7.1.2.1.1. What methods? (Other)

You selected "Other". Please provide what methods you used to remove cheetah

---

7.1.2.1.1. Estimated total cheetah relocated alive in the past five years (Relocation)

Please provide an estimate of the total amount of live cheetah relocated from your land

---

7.1.2.1.2. Estimated total cheetah deaths during relocations

Please provide an estimate of the total amount of cheetah that died during relocation from your land

7.1.2.2. Estimated total cheetah removals using lethal methods

Please provide an estimate of the total amount of cheetah removed from your land

7.1.2.3. Do you keep a record of removed cheetah?

Please provide if you have an up to date record of removed cheetah

- Yes
- No

7.1.2.3.1. What are the numbers of males?

You selected "Yes". Please provide the numbers of male cheetah that have been removed from your land

7.1.2.3.2. What are the numbers of females?

You selected "Yes". Please provide the numbers of female cheetah that have been removed from your land

7.1.2.3.3. What are the numbers of cubs?

You selected "Yes". Please provide the numbers of cheetah aged 0-2 that have been removed from your land

7.1.2.3.4. What are the numbers of sub-adult individuals?

You selected "Yes". Please provide the numbers of cheetah aged 2-4 that have been removed from your land

7.1.2.3.5. What are the numbers of adult individuals?

You selected "Yes". Please provide the numbers of cheetah aged >4 that have been removed from your land

7.1.2.3.6. What are the numbers of collared individuals?

You selected "Yes". Please provide the numbers of collared cheetah that have been removed from your land

7.1.2.3.7. What are the numbers of recurring individuals?

You selected "Yes". Please provide the numbers of cheetah that have been removed from your land but came back

8. Farming activity opinions

8.1. Welfare *Please provide how you feel about these welfare statements*	Strongly disagree	Disagree	Neutral	Agree	Strongly agree	No comment
My farming practises are important for my economic well-being	○	○	○	○	○	○
Human-wildlife conflict affects my farming activity	○	○	○	○	○	○
I get sufficient support from the government	○	○	○	○	○	○
If I got more support, I would be more tolerant to predators	○	○	○	○	○	○

8.2. How would you like to be supported?

Please provide what kind of support you would want and need to farm effectively

9. Farming views and research

9.1. Past and currect research

Please provide if you have had any research taken place about carnivores on your farmland in the past or currently and what was it about

9.2. Coexistence with and utilizing free-roaming cheetah

Please provide how you believe we can achieve coexistence with and utilization of free-roaming cheetah

Appendix II

FRCC Questionnaire Predator Recognition

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8

STANDARDIZED PROTOCOL FOR CAMERA TRAPPING & SCAT COLLECTION

This SOP is created to establish a standardised, effective and systematic way of camera trap analysis and scat collection. By creating one effective protocol, the procedure becomes more smooth (i.e. new people/staff can understand quickly how the work is done and all results are similar and therefore more useful in analysis). This SOP can, and should, be used at all times.

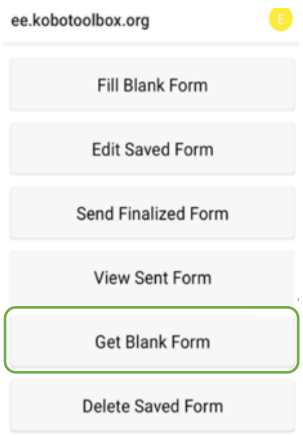
All fieldwork information needs to be stored in Kobo. This can be done via the KoboCollect app (Android phones) or the following https://ee.kobotoolbox.org/x/pLf4hGos (iPhones).

GETTING STARTED WITH KOBOCOLLECT (Android)

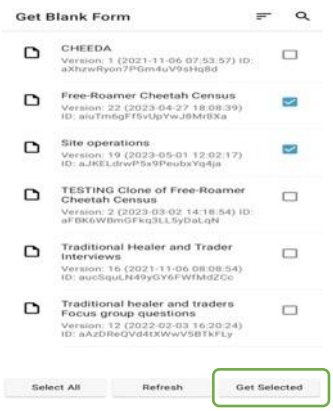
1. Install the KoboCollect app (https://play.google.com/store/apps/details?id=org.koboc.collect.android)
2. Open the app
3. Click on "Configure with QR code"
4. Scan the code provided.
5. Click on "Get blank form" (see below).
6. Make sure only "free-roamer cheetah census" and "site operations" are ticked (untick the others) and click "Get selected" at the bottom right of the screen (see below).
7. When filling in a new survey, click on "fill blank form" and pick which survey you want to use; free-roamer cheetah census for the questionnaire and site operations for any camera trapping or scat collection.

If any of the forms are updated, it will be mentioned in the "FRCC – Questionnaires" groupchat. In order for these updated forms to load into KoboCollect, please re-do the steps from step 5 onwards.

STEP 5



STEP 6



Before entering a grid cell, arrange a form of contact with the person who worked in that cell before you (if you are not the first) to get a full update on camera locations, contact people, etc. Make use of the dedicated WhatsApp groups to communicate

EQUIPMENT

During the pre-planning phase, the equipment used during camera setup at a sentinel site should be checked and serviced to ensure all tools are in working order before going into the field. Below is a detailed checklist of the equipment needed to setup camera stations in one grid cell:

1. 8-10 camera traps (minimum) per cell (6 to remain behind, 4 to be used as recce cameras)
2. 10-12 16gb Memory cards (minimum), including small SD card label sticker or a permanent marker to label them when needed
3. Small boxes to store SD cards when they are not used in traps
4. Cable ties (long and fat ones) or adjustable straps/security cables with locks or even a security box for the camera traps
5. 8-10 metal stakes or attachment poles to place the traps on when natural features aren't available
6. A device to review trapping photos on in the field (phone, laptop)
7. Fully charged batteries to run the camera traps, including 2 spare sets of batteries
8. A battery charger (if using rechargeable) and battery tester
9. Hand-held GPS unit, including spare batteries
10. A cloth to dry/clean the camera trap
11. Paper and permanent marker(s) to make the "start" and "end" images
12. Permanent markers, pens and pencils
13. Toolkit, including: pliers, knife, foldable saw, grass slasher, lighter, branch cutter, hammer

CAMERA LABELLING AND SETTINGS

There is a large number of cameras and SD cards being used in this study across a vast area; thus great care must be taken to ensure each camera is correctly labelled to allow for easy identification of images to sites, knowing precisely how many and which cameras are in the field, as well as effortlessly locating the cameras and replacing the correct camera when the time comes.

A camera trap and an SD card will both have a unique, 3-digit number (e.g., 003). This number will forever stay the same. The number on the camera is independent from the SD card, meaning that camera 001 can be paired with SD 025. Camera and SD card numbers should **NEVER** be reused.

Before deploying a camera trap, check if all the labelling is present and correct. There needs to be a label on the outside of the camera, on the inside of the camera (if it opens) and at the front of the SD card. The label on the outside of the camera needs a piece of transparent tape to protect it from weather conditions.

If a camera or SD card is not labelled yet, please do so accordingly. Most cameras should be labelled before they arrive at the fieldworker, if not contact Marna or Nynke to check which labelling can be added on the cameras.

Before activating a camera **for the first time**, some steps are required to get the settings correctly. The following steps are based on the Spartan cameras but should be followed us much as possible when using other cameras:

1. Start with changing the date and time. Set the date format first, this will make sure we have the DD/MM/YY format. After this change the clock to the current date and time. If a camera cannot have the DD/MM/YY format, add this on the label. This way, when someone checks the camera, that person knows how to read the date.
2. Set the trigger sensitivity. Set the sensitivity to high with a trigger interval as close to 0 seconds as possible (but not 0). With the Spartan cameras, this will be 5 seconds.
3. Set the temperature to the correct scale. Change the Temperature Scale, which will probably be at Fahrenheit, to Celcius.
4. When a camera trap makes use of a beeping sound, remove this. This can often be found under the "System" section.
5. Make sure that if the camera has an "overwrite" function, it is turned off to prevent new images overwriting the older ones.
6. Lastly do a main menu check. Check if the SD card has enough space, if the temperature is set correctly, if the batteries are full and if the timestamp is correct.

All other types of cameras will have settings close to the Spartan camera. Therefore checking date, time, trigger sensitivity, temperature and overwrite is mandatory with every camera model.

IDENTIFICATION OF SENTINEL SITE LOCATIONS

Camera trapping starts by looking for sentinel sites. These sites mostly consist of play trees, fence lines, waterholes or animal crossings. Around these areas, cheetah often leave marks such as scat, spoor or scratches. Once a site is confirmed to be used by cheetah, camera traps are placed. After the placement, camera traps need to be checked regularly to prevent the loss of data. This can happen due to camera failure, overheating, knock-downs, empty batteries or theft (both animal and human). The next pages will explain how this is done.

KEY CONCEPTS AND ESSENTIAL POINTS

While the project is still expanding on the existing knowledge of hot spot connectivity, we cannot afford to waste time in cells as there are just too many to cover over a short period of time In order for this monitoring network to cover enough ground and operate continuously for 2 - 3 years, we will have no more than three sentinel sites (2 paired cameras each) per cell. Any additional cameras can be used as recce cameras. Recce cameras can be deployed for 2 to 3 weeks at minimum, thereafter the 3 best sentinel sites for permanent deployment should be chosen, ensuring 3 sites per grid cell and 1 site per quarter grid cell (quadrant).

Please note – you do NOT have to find cheetah on your cameras in order for the cell to be activated. You are only required to do due diligence and choose the best sentinel sites. This can also consist of sites with high activity of other wildlife. It is ideal to find cheetah, but they are not always going to be at the site. Thus choose the best sites, standardise effort, and proceed to the next cell.

PRIORITIES WHEN DECIDING ON SENTINEL SITES FOR CAMERA DEPLOYMENT:

1. MARKING TREES/PLAY TREES

Marking/play trees are important communication points for cheetahs. They scratch the tree, scrape the ground with their hind legs and use urine and faecal matter to leave visual and olfactory signals to other cheetahs in the area.

- Consult with locals: If you are in an area where cheetahs are known to live, it may be helpful to consult with locals such as farm workers and landowners. They may be able to provide more specific information about where to find sentinel marking trees. If possible, combine this with the execution of a survey as well.

- Evidence of marking: Such as claw marks or urine stains. Cheetahs often scratch the bark of trees with their claws and leave scent marks with their urine. Old scat present around the base of the tree and in the horizontal branches are a clear indicator that the tree is being used as a sentinel marking tree.

- Prominent branches and structures such as termite mounds, downed fence posts, energizer boxes, fallen trees, etc: Cheetahs prefer to mark trees with prominent branches that are easy to climb and provide a good vantage point. Look for trees with large, horizontal branches that could support a cheetah's weight. If you can climb and stand on it, so can a cheetah. If you locate a fallen tree, look at the treetop. If the leaves are still on and look luscious, it is a newly fallen tree and is likely not (yet) in use as a sentinel site by cheetah.

2. FENCE CROSSINGS

Fence crossings are important locations as they allow for cheetah to cross between properties and transverse the landscape. These crossings can be identified as holes in fences that have clear and well-formed animal pathways leading to and from them. Local knowledge from farm workers and landowners will be useful to identify these areas on the property perimeter. Cheetah spoor and evidence of fur in fence wire are also indicators that cheetah use these crossings and pathways.

3. FIXED WATERHOLES & STRUCTURES (boreholes, pumps, windmills, energizer boxes)

Permanent water sources are not ideal for cheetah, partly because they don't have such serious water demand. But they do count as communication hubs for many species, so in the absence of better locations, good coverage of a permanent water point is a suitable option per quadrant.

What to do if no sites are found/identified?
If an area does have appropriate trees or fence holes, but no cheetah signs are present, a camera trap pair can still be placed. Data without cheetah is still valuable in order to have a certainty of cheetah absence in that area. However, if no suitable sites are found (i.e. no appropriate sentinel sites, no sign of cheetah **and** no other animal activity present), do not place a camera and note it as a dead zone. Because there is no standardised way to check those quadrants or entire cells, we will revisit these sites with a more appropriate technique.

LABELLING OF SENTINAL SITES

When a sentinel site has been found, the site is given a number. It is important to give every site a number, even when no camera traps are put up. For example, site 001 is a good site but you did not put cameras. After visiting 3 more sites – 002, 003 and 004 – you decide to come back to site 001 to put up cameras. For the camera activation, make sure you then use site 001 and not site 005. In order to keep track of site numbers, it is recommended to make notes while you're out. This can be done either in a notebook or, preferably, on a handheld GPS or WhatsApp where you can also add the location.

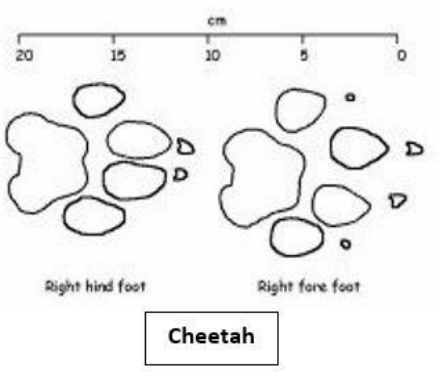
When entering information in the KoboCollect app (preferred when you can) or (https://ee.kobotoolbox.org/x/pLf4hGos (offline version) all identified sentinel sites in each individual grid cell must be labelled according to the grid cell code (e.g. NW_D4), followed by 001, 002, 003, etc. (e.g., NW_D4_001; NW_D4_002; NW_D4_003). As soon as you move to a new grid cell, you start labelling from 001 again (e.g. NW_E4_001; NW_E4_002;NW_E4_003).

SPOOR IDENTIFICATION

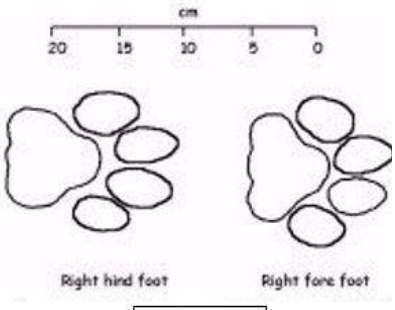
When looking for sentinel sites as above, key identifiers will be scat and spoor. When found, both must be documented.

When identifying spoor, make use of the following steps. Use the images below and the ones provided within the KoboCollect app (preferred) or via the Kobo link to help identify cheetah spoor from other predator spoor.

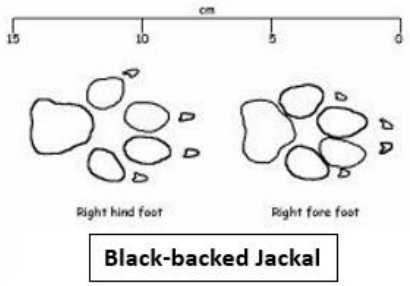
1. Identify spoor as being from a cheetah: Use visual schematics and images below to aid in identifying the spoor. Look for other signs such as scat and markings to ensure the spoor is not from another predator.
2. Look for claw marks above the spoor, this can help distinguish between cheetah and other feline spoor. Please use below images for comparisons.



Cheetah



Leopard



Black-backed Jackal

SCAT COLLECTION

When collecting scat, make use of the following steps. Use the images below and the ones provided within the KoboCollect app (preferred) or via the Kobo link to help identify cheetah scat from other predator scat. Information from these scat collections will be used to determine which prey species are being consumed in these areas.

Instructions

1. Identify scat as being from a cheetah: Use visual schematics and images below to aid in identifying the scat. Cheetah scat are generally in the region of 5 – 10cm long but can vary (anything smaller than 5cm should not be collected as these could be from other species), and could also have hair material in it when opened.
2. Confirm with track/spoor nearby, and collect. Scat without track confirmation of species nearby must be noted and will be listed down the priorities list.
3. Collect fresh scat and place into a paper envelope. Try and avoid using plastic bags as it will retain too much moister in this damp environment and cause the scat to 'rot'. If you however have no choice, move it to a paper bag ASAP.
4. Staple the envelope shut, but keep a small opening so that the scat can 'breathe'.
5. Write the scat collection number (on the envelope in **PENCIL** (ink may run due to moisture from the scat). All collected scat in each individual grid cell must be labelled according to the grid cell code (e.g. NW_D4), followed by SS001, SS002, SS003, etc. (e.g., NW_D4_SS001; NW_D4_SS002; NW_D4_SS003). As soon as you move to a new grid cell, you start labelling from 001 again (e.g. NW_E4_SS001; NW_E4_SS002;NW_E4_SS003).
6. Record information in KoboCollect (preferred) or via the Kobo link.
7. Dry scat in envelope in the sun for at least two days.

SCATOLOGY

The below images show cheetah scat next to certain objects for scale. Note the thickness and short length in each image.

Cheetah Scat with Matchbox

Cheetah Scat with Carabiner

Cheetah Scat with Radio

Leopard scats are large in size, with scats reaching 25cm in length and a width of 3.7cm. Grass and fine plant material are characteristics of leopard scat, together with it being elevated sometimes. Please observe the image with leopard scat placed next to a radio. Note the length (over 10cm) and the smaller width.

The next images is of a serval scat with a radio for scale. Note the thin segmented appearance and small size of the scat in comparison to the cheetah scat. The last two image to the right show a small domestic dog scat (3rd image) next to a radio for scale, and a large domestic dog scat (image on the far right) next to a radio for scale.

Leopard Scat with Radio

Serval Scat with Radio

Small dog Scat with Radio

Large Dog Scat with Radio

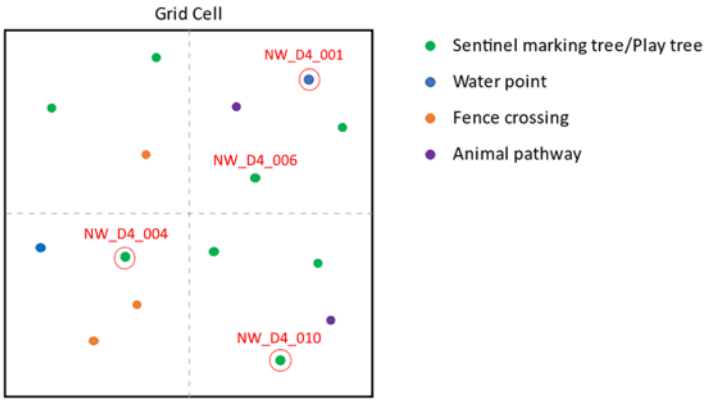
GENERAL CAMERA TRAP LAYOUT AND SETUP PLAN

Within each grid cell, points of interest must first be identified across the landscape before camera traps are set. When leaving a cell, three permanent sentinel sites are required to have active camera pairs. To ensure these are spread over the area effectively, each grid cell is further divided into quarters (4 quadrants). At least three of these four quarters must have an active sentinel site with 2 paired cameras so that we cover as much area as possible.

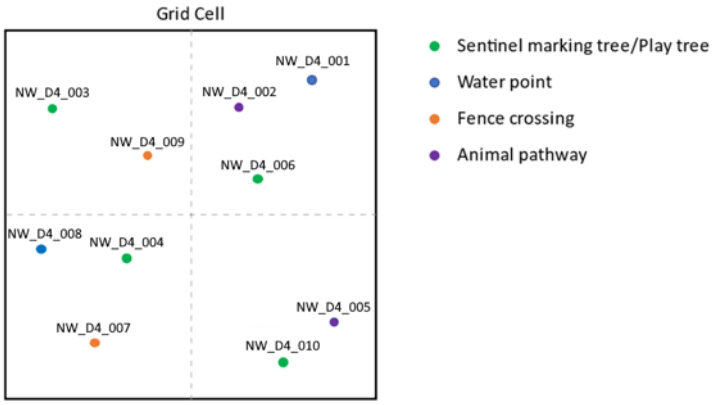
All placed cameras (recce & permanent) should ideally be checked within 72 hours of placement to make sure the angle is correct, the cameras still work, no drop-down has happened, etc. This improves the success rate and quality of the photos. Recce cameras should be checked again after 2 – 3 weeks to establish if it's a viable sentinel point (i.e. a lot of general wild animal activity such as cheetah, jackal, porcupine, leopard, caracal, antelope, etc.). If no animal activity is detected during this location trial time, the cameras must be deactivated and retrieved.

<u>Here an example of grid cell D4 in the North West province:</u>

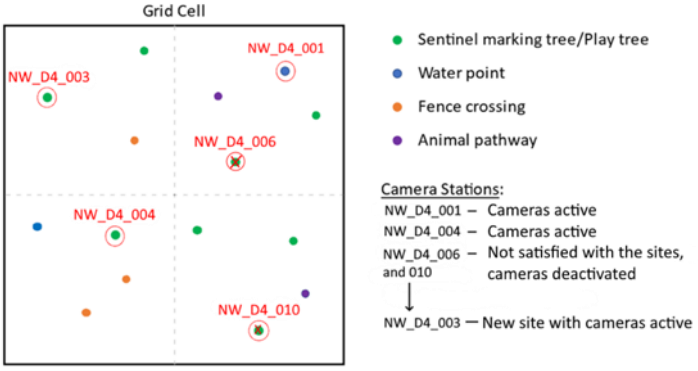
Here you see that within grid cell D4 in the North West province, 10 sentinel sites have been identified. Cameras can be placed either immediately after identifying a sentinel site, or later on. However, the sentinel site number should always stay the same (see next image).



This image shows that camera traps have been placed at site 001, 004, 006 and 010. The sentinel site numbers stay the same as before.



Three sentinel sites must keep their paired recce stations, which will then turn into permanent sites. In this case, site 006 and 010 did not get sufficient data, both sites will be removed. In order to have 3 permanent sites, one set of cameras has to be put up somewhere else within the grid.

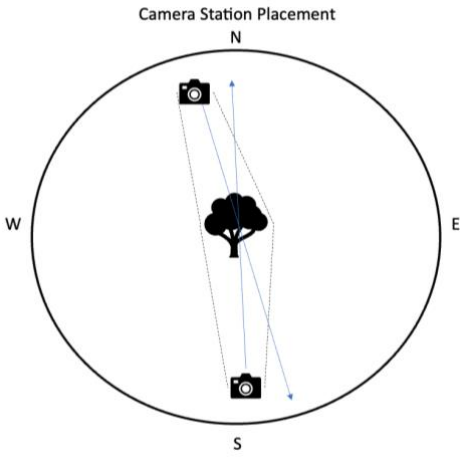


NOTE: the sentinel site number does not move to a new location. Instead, the number that was previously allocated to the site (NW_D4_003) when it was just a sentinel site without cameras is being used again. If it was a site that hasn't been identified before, it would receive a completely new number (e.g. NW_D4_011).

CAMERA STATION ACTIVATION

When a sentinel site has been found, camera traps can be placed and activated immediately. This site should have 2 activated camera traps before you leave. Setting up a camera trap at a sentinel site should follow the following steps.

1. Once you have identified the sentinel site, choose a north-south bearing for the cameras that provides a good view of the site and the surrounding area. One camera should be facing south and the other north. Make sure the cameras are positioned at a suitable height (at least 1m from the ground to ensure it is out of reach of animals) to capture the cheetahs as they mark. The cameras must be at a slight angle and not face each other directly as the flash from each camera can interfere with the picture quality. See image →



2. Clear the area of long grass and other vegetation that could activate the camera. Ensure all dead or hanging branches are cleared to prevent false images. This also goes for material on the ground. The cleared area will also form some protection in case of fire.

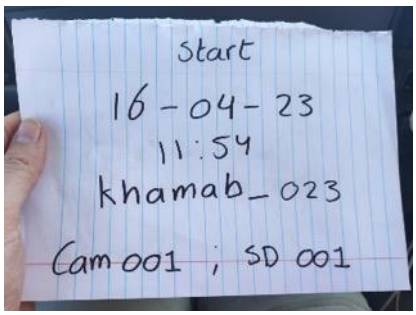
3. Install each camera on a metal stake or preferably on natural material (e.g. a tree). When using a stake, ensure a quarter of the stake is in the ground so that it is secure and will not be easily pushed over. Attach a camera with cable ties or secure strapping to the stake. Make sure the camera is securely fastened to prevent it from falling or being knocked over.

4. Set up the camera trap: It is essential that the **date and time is correct and make it DAY/MONTH/YEAR**. Make sure that its set on **high sensitivity** – if it triggers because of grass, then change position or clear the site better. Ensure it's set on **multiple photos (no video)** and **set time between captures as low as possible**. Smallest window with maximum amount of shots. Make sure that the **"overwrite" function is turned off**. When switch back from setting mode, make sure to switch it on.

5. Fill in the KoboCollect app (preferred) or the Kobo link with all prompted information.

6. Walk in front of the camera to test its motion detection. Remember to stand at the base of the tree or next to the branch of interest to ensure the camera is capturing the area sufficiently. Use a tablet/camera/laptop to check the images and adjust the camera trap accordingly. Believe it or not – walk in front of the camera like a cheetah. When you use cameras that are equipped with a screen, you can check the footage while walking in front of the camera. Adjust angle when needed.

7. Activate the camera and take a 'start' photo. This photo should contain a paper, whiteboard of chalkboard with the action (i.e. "start" when it's the first activation, "re-activate" when you activate it again due to e.g. fall down, "end" when taking the camera down), date, time, sentinel site number, camera number (e.g. 001) and SD card number on it. Check the camera to ensure the images of the board are clear.



8. Ensure all equipment is collected as well as any rubbish (cable tie cut offs, plastic etc).

NOTE: after the cameras have been activated, do not go in the frame to do tasks such as clearing the area, removing cages etc. Please ensure that the camera is off at those times to prevent unnecessary images.

NOTE: do not do your "business" in close proximity (<100m) of the sentinel site. This will influence the wildlife.

CAMERA CHECK AND RETRIEVAL

The cameras should be checked regularly in case of failure, knock-down, empty batteries or theft. The first check should ideally take place within 72 hours after activation. Recce cameras on sentinel sites that have not been transformed into permanent sites, should ideally be checked again after 3 - 4 weeks of activation to decide whether they become permanent or not. If no activity of cheetah has been seen at the site after 3 – 4 weeks, remove the recce cameras. All permanent sites should be checked every 6 to 8 weeks. Do not check cameras every week, this will disturb the wildlife. If you still have camera trap(s) available, bring them with you in case of a malfunctioning/broken camera. This way the camera can be replaced immediately.

When arriving at the sentinel site for a check:

1. Check if date and time, and other settings are correct before switching off the camera trap.
2. Download images and empty the memory card. Store the images on a hard drive and label the folder as the grid cell you're in. Place the 2 folders (one for each camera trap on this site) within this grid cell folder and name them as follows: Camera_SD_checkdate (e.g. Cam034_SD025_29-04-23)
3. Check the batteries (with the battery tester) and change the batteries when necessary.
4. Purpose of the check is to see if the camera still works properly and to confirm whether it's a sentinel site or not. If there is a lot of activity from different species, make a call to use it as a permanent site. If no activity is seen, decide on whether or not to continue with this site as an option.
5. If a camera is broken or malfunctioning, make sure to replace it with a new camera and fill in the "camera activation" in the KoboCollect app (preferred) or the Kobo link with all prompted information.
6. Check to see if images are well framed, whether there is vegetation in the way, whether there is a problem with sunlight or the opposing camera flash causing blank photos or to detect possible camera trap failure (e.g., black picture because of non-functioning flash).
7. Check if both camera traps have approximately the same number of pictures. If there is a marked discrepancy, check the sensitivity of the motion detector of the camera trap with the lowest number of pictures and if necessary, replace the camera.
8. After checking the camera trap, the orientation of the camera traps should be readjusted and tested when necessary. Remember to stand at the base of the tree or next to the branch of interest to ensure the camera is capturing the area sufficiently. Use a tablet/camera/laptop or camera trap screen to check the images and adjust the camera trap accordingly.
9. When (re-)activating the camera, take a 'start' or 're-activation' photo. This photo should contain a paper with the action, date, time, sentinel site number, camera number (e.g. 001) and SD number on it. Check the camera to ensure the images of the board are clear.
10. Fill in all the information in the KoboCollect app (preferred) or via the Kobo link.
11. Before leaving the site ensure all cameras are activated, secured and live. Ensure all equipment is collected as well as any rubbish (cable tie cut offs, plastic etc).

When arriving at the sentinel site for a retrieval:

1. Cameras have to be retrieved in pairs (the complete site).
2. Check if date and time, and other settings are correct before switching off the camera trap.
3. Download images and empty the memory card.
4. Store the images on a hard drive and label the folder as the grid cell you're in. Place the 2 folders (one for each camera trap on this site) within this grid cell folder and name them as follows: Camera_SD_retrievaldate (e.g. Cam034_SD034_29-04-23)
5. Fill in all the information in the KoboCollect app (preferred) or via the Kobo link.
6. Before leaving the site ensure all cameras and poles are retrieved. Ensure all equipment is collected as well as any rubbish (cable tie cut offs, plastic etc).

NOTE: when doing something off protocol regarding the camera trapping, please add this in a note or description section within the Kobo survey or send a message.

Site operations

1. General

1.1 Name

Specify who adds this record, preferably first and last name

1.2 Enter the current date and time

yyyy-mm-dd hh:mm

1.3 GPS coordinates

Add the gps coordinates of the point of action

latitude (x.y °)

longitude (x.y °)

altitude (m)

accuracy (m)

1.4. Grid cell

Provide the grid cell where the action will be performed (e.g. LP_E11). If needed you can use the maps: NW, NC, LP

1.5 Farm name

1.6 Which operation will take place?

Multiple answers possible

- [ ] Sentinel site identification
- [ ] Camera/site activation
- [ ] Camera check
- [ ] Site retrieval
- [ ] Scat collection
- [ ] Spoor identification
- [ ] Kill identification
- [ ] Trap set up
- [ ] Trap check

1.6.1. Sentinel site number

Please add a number to create an individual ID for this site (i.e. grid+number: NW_D4_001)

1.7. Are there predator signs in the area?

- ◯ Yes
- ◯ No

1.7.1. Presence of markings

Multiple answers possible

- ☐ Scat
- ☐ Scratches
- ☐ Spoor
- ☐ Other

Is the found scat cheetah scat?

If the observed scat is cheetah scat, please tick the "scat collection" box in the previous question

---

Is the found spoor cheetah spoor?

If the observed spoor is cheetah spoor, please tick the "spoor identification" box in the previous question

---

2. Sentinel site identification

2.1. Site description

- ☐ Marking tree
- ☐ Animal crossing
- ☐ Fence line
- ☐ Waterpoint

2.1.1. Tree type

State the type of the play tree

2.1.2. Take a photo

Please use your phone to take a photo of the tree

Click here to upload file. (< 10MB)

2.1.1. What type of fence?

Specify the type of fence

- ◯ Small (2-4) strands
- ◯ Game proof
- ◯ Predator proof

2.1.1. What type of waterpoint?

Specify the type of waterpoint

- Trough
- Water hole
- River

2.2. Camera trap placed?

State if a camera trap is placed at this sentinel site

- Yes
- No

2.3. Description

If necessary, add extra information on the sentinel site

---

3. Camera/site activation

3.0. Is it a recce site or a permananent (paired) site?

- Recce
- Permanent

» 3.1. Camera number 1

3.1.1. Camera number

Specify the number of the camera trap (e.g. camera 001)

3.1.2. SD card number

Specify the number of the SD card (e.g. SD 001)

3.1.3. Which direction is the camera facing?

- North facing
- South facing

3.1.4. Camera settings

Specify the trigger interval of camera 1

- 1 second
- 3 seconds
- 5 seconds

3.1.5. Are you placing another camera?

- Yes
- No

» 3.2. Camera number 2

3.2.1. Camera number

Specify the number of the camera trap (e.g. camera 001)

3.2.2. SD card number

Specify the number of the SD card (e.g. SD 001)

3.2.3. Which direction is the camera facing?

- North facing
- South facing

3.2.4. Camera settings

Specify the trigger interval of camera 2

- 1 second
- 3 seconds
- 5 seconds

4. Camera check

» 4.1. Camera number 1

4.1.1. Camera number

Specify the number of the camera trap (e.g. camera 001)

4.1.2. SD card number

Specify the number of the SD card (e.g. SD 001)

4.1.3. Was the camera working?

Specify if camera 1 was working when checked

- Yes
- No

4.1.3.1. What could be the reason?

State possible reason(s) for the camera not working

4.1.3.2. Are you replacing the camera?

- Yes
- No

» » Replacement camera 1

4.1.3.3. New camera number

Specify the number of the new camera trap (e.g. camera 001)

4.1.3.4. New SD card number

Specify the number of the new SD card (e.g. SD 001)

4.1.3.5. Which direction is the new camera facing?

- North facing
- South facing

4.1.3.6. Camera settings

Specify the trigger interval of the new camera

- 1 second
- 3 seconds
- 5 seconds

4.2. Write down any necessary information (e.g. camera fell down)

4.3. Is camera 2 being checked?

- Yes
- No

» 4.4. Camera number 2

4.4.1. Camera number

Specify the number of the camera trap (e.g. camera 001)

4.4.2. SD card number

Specify the number of the SD card (e.g. SD 001)

4.4.3. Was the camera working?

Specify if camera 2 was working when checked and/or retrieved

- ◯ Yes
- ◯ No

4.4.3.1. What could be the reason?

State possible reason(s) for the camera not working

_____

4.4.3.2. Are you replacing the camera?

- ◯ Yes
- ◯ No

» » Replacement camera 2

4.4.3.3. New camera number

Specify the number of the new camera trap (e.g. camera 001)

_____

4.4.3.4. New SD card number

Specify the number of the new SD card (e.g. SD 001)

_____

4.4.3.5. Which direction is the new camera facing?

- ◯ North facing
- ◯ South facing

4.4.3.6. Camera settings

Specify the trigger interval of the new camera

- ◯ 1 second
- ◯ 3 seconds
- ◯ 5 seconds

4.5. Write down any necessary information (e.g. camera fell down)

_____

5. Site retrieval

5.1. Reason for retrieval

- ◯ Removal/rotating process (>100 days)
- ◯ High theft area
- ◯ Other

5.1.1. What other reason for removing the site?

» 5.2. Camera number 1

5.2.1. Camera number

Specify the number of the camera trap (e.g. camera 001)

5.2.2. SD card number

Specify the number of the SD card (e.g. SD 001)

5.2.3. Write down any necessary information

5.2.4. Is camera 2 retrieved as well?

- ◯ Yes
- ◯ No

» 5.3. Camera number 2

5.3.1. Camera number

Specify the number of the camera trap (e.g. camera 001)

5.3.2. SD card number

Specify the number of the SD card (e.g. SD 001)

5.3.3. Write down any necessary information

6. Cheetah scat collection

6.1. How many scats are you collecting from this site?

Please provide a number of the amount of scats you will be collecting at this location

Please compare all the scat found with the images provided to prevent mistakes

---

6.2. Scat number

Specify the scat number (e.g. S001). If you have multiple scats, provide the numbers as follows: S001; S002

6.3. Scat location description

Specify where the scat was found. In case of multiple scats, you can choose multiple options.

- [ ] In a tree
- [ ] In a bush
- [ ] On open ground
- [ ] Other

6.4. Scat sighting

Use your camera to take a photo. If you have multiple scats, place them in an image together with the scat bags incl numbers visible besides each scat sample

Click here to upload file. (< 10MB)

6.5. Scat description

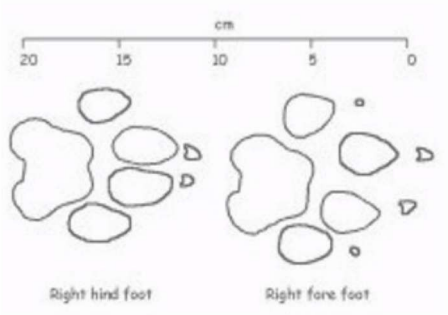
Write down any information regarding the scat

6.6. Scat confidence

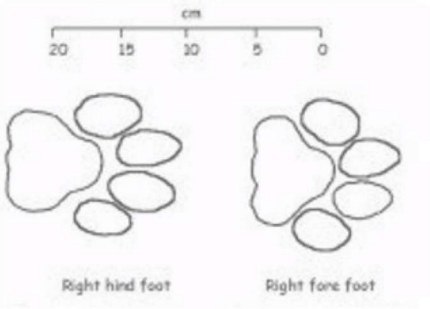
	Uncertain	Neutral	Confident
How certain are you?	○	○	○

7. Cheetah spoor identification

Please compare all the spoor found with the images provided to prevent mistakes



Cheetah



Leopard

7.1. Spoor number

Specify the spoor number (e.g. SP001)

7.2. Spoor location description

Specify where the spoor was found. Multiple answers possible

- [ ] At a tree
- [ ] Open ground
- [ ] Road
- [ ] Other

7.3. Spoor sighting

Please use your phone to take a photo of the spoor

Click here to upload file. (< 10MB)

7.4. Spoor confidence

	Uncertain	Neutral	Confident
How certain are you?	◯	◯	◯

7.5. How long was the track in meters?

Provide the length between the first and last step

7.6. Can multiple individuals be distinguished in the track(s)

- ◯ Yes
- ◯ No

7.7. What individual(s) made the track(s)?

Multiple answers possible

- [ ] Adult
- [ ] Young

7.8. Any remarks

Please provide any information that you feel is necessary

---

8. Kill identification

8.1. Type of prey

Specify the species that has been killed

8.2. Age of the prey individual

Estimate the age in years

8.3. Sex of the prey individual

- ◯ Male
- ◯ Female

8.4. Kill location description

Specify where the kill was found

- [ ] In a tree
- [ ] In a bush
- [ ] Open ground
- [ ] Road
- [ ] Other

8.5. Is cheetah ID known for this kill?

- ◯ Yes
- ◯ No

8.5.1. Provide the ID

8.6. Where was the carcass opened?

Multiple answers possible

- [ ] Armpit
- [ ] Back
- [ ] Groin
- [ ] Neck/head
- [ ] Ribs
- [ ] Rump
- [ ] Stomach
- [ ] Other

8.7. Are bite marks present on the body?

- ◯ Yes
- ◯ No

9. Trap set up

9.1. What kind of trap is set up?

- ◯ Normal cage trap
- ◯ Smart trap
- ◯ Other

9.2. Was anything used to attract/lure a cheetah to the trap?

- ◯ Yes
- ◯ No

9.2.1. What what used as a lure?

_____

How often do you plan to check up on the trap?

Please provide if youll check up hourly, daily, every two days, weekly, etc.

_____

10. Trap check

10.1. What was in the trap?

- ◯ Cheetah
- ◯ Other animal species
- ◯ False trigger
- ◯ Nothing

10.1.1. What other animal species?

10.2. Was the animal alive?

- Yes
- No

10.2.1. What could be the cause of death?

10.2.2. Mortality sighting

Use your camera to take a photo of the deceased individual

Click here to upload file. (< 10MB)

10.2.1. Was the animal released successfully?

Please specify if the other animal species (not cheetah) was released successfully

- Yes
- No

10.3. What drug was used to immobilize the cheetah?

10.4. Who did the darting of the cheetah?

10.5. What type of collar was placed on the individual?

10.6. What is the code of the collar placed on the individual?

10.7. What genetic code is associated with the individual?

Please specify the genetic code given to the blood that was drawn from the individual

10.8. Left flank

Use your camera to take a photo of the left flank of the cheetah

Click here to upload file. (< 10MB)

10.8. Right flank

Use your camera to take a photo of the right flank of the cheetah

Click here to upload file. (< 10MB)

10.9. When will you release the individual?

- The same day
- The next day
- Other

10.9.1. Where do you keep the individual?

10.9.1. Specify "other"

10.10. Site of release

Specify where you have released the individual

10.11. Is the trap retrieved?

- Yes
- No

Please wait with closing the browser until the survey is submitted. If there is no available internet, open the browser again when internet is present.

Free-roaming Cheetah Census Camera Trap Assessment

1 Introduction

Camera trapping is an essential and widely used method for wildlife monitoring and ecological research, providing non-invasive insights into species distribution, behaviour, and population dynamics [1][2]. However, the reliability of camera trap data depends on several factors, including the quality and placement of the cameras, environmental conditions, and post-processing accuracy [7]. This overview presents a systematic assessment of the quality of eight different camera trap types: Browning (model RTC-5HDX), Bushnell (models 119932C, 119936C, 5710), Cuddeback (model 1279), Panthera (model V7), Solar powered (models Ceyomur, Solaris, and Wi-Fi), Spartan (model SR3-CX), and Spartan (model SR4-BK). Spartan is the only camera type with model distinctions within this overview due to a high usage of model SR3-CX during the Free-Roaming Cheetah Census (FRCC; N = 139). The camera traps used during the census' field period (Oct. 2022 – Dec. 2025) were placed with the aim to gain reliable estimates for the wild South African free-roaming cheetah (*Acinonyx jubatus jubatus*) population. The camera placement focused within the South African cheetah IUCN range [3], in the provinces Northern Cape, North-West and Limpopo.

2 Methodology

A total of **389** camera traps were deployed across the study area (table 1). Cameras were positioned at a height of ~1.60m meters to prevent predatory damage, set to medium sensitivity, and placed to capture cheetah (e.g. play trees, trails, water sources and fence lines). The camera quality assessment was conducted by reviewing ~5 million images in TrapTagger, an open-source AI-assisted web application with a specialized southern African classifier [8], and evaluating them based on the following criteria:

Table 1: The different camera trap models used for the FRCC. The Vista Trail and Rik rhino cameras were not used in this analysis due to small sample size.

	Camera Model	*N*
1	Browning BTC-5HDX	7
2	Bushnell 5710	3
3	Bushnell 119932C	4
4	Bushnell 119936C	6
5	Cuddeback 1279	58
6	Panthera V7	31
7	Rik Rhino BG583	3
8	Solar Ceyomur CY95	60
9	Solaris Weapon 4K	20
10	Solar Wifi	25
11	Spartan SR3-CX	139
12	Spartan SR4-BK	32
13	Vista Trail	1
		389

1. **Species Identifiability**: Determination of whether the captured images allow for clear identification of species (i.e. no over- or underexposure, blur, haze).
2. **False Triggers**: Calculation of the proportion of images triggered without the presence of wildlife.
3. **Sensitivity**: Assessment of the sensitivity of each camera type by comparing the total number of images across the days active
4. **Failure rate**: Assessment of the quantity of camera breakage due to internal/software issues (e.g. screen or camera not turning on, flash not working, pink/yellow glow, unreliable sensors, error messages and firmware issues).

5. **Camera performance**: Determination of the number of days a camera should have taken images versus the number of days it was active (randomly shutting off, not triggering, quick battery depletion)
6. **Image quality**: Oral assessment of how easy the annotators could identify wildlife, how they perceived the image clarity, and if day and night quality differences are observed.

For all analyses, Rik rhino and Vista Trail cameras are excluded due to a low sample size (N=3, N=1 respectively).

3 Results

Approximately 5 million images were used for all analyses in this overview, ranging from 101,888 images from all Panthera cameras up to 2,248,669 images from the Spartan SR3-CX cameras.

3.1 Species identifiability and false triggers

A Shapiro-Wilk test [9] was conducted to assess the normality of the distribution of images with a wildlife, unknown (species identifiability), or none (false triggers) annotation for all camera types. The non-normality (p<0.001) resulted in the use of a Kruskal-Wallis test [6] to evaluate statistical differences among camera types.

Kruskal-Wallis rank sum tests revealed highly significant differences between the seven camera types in species identifiability (unknown images; p<0.001) and false triggers (wildlife versus empty images; p<0.001). A Dunn's test [5] was used with a Holm-Bonferroni correction to identify the specific pairwise differences between camera types. The Holm method is applied when analysing multiple comparisons (i.e. seven different camera types), as more comparisons equals a higher probability of obtaining false positives, by adjusting the p value upwards making it more difficult to stay under the regular 0.05 threshold [4]. For wildlife capture, most pairwise comparisons were significant, with some exceptions: Bushnell-Cuddeback, Bushnell-Panthera, Cuddeback-Spartan SR4-BK, and Solar-Spartan SR3-CX showed no significant differences (p.adj > 0.05). Bushnell-Panthera was also the only non-significant pairwise comparison for false/empty image capture (p.adj > 0.05), all other camera types differed significantly from each other. Unknown image capture showed more distinct differentiation, with both Spartan models having significantly less unidentifiable images compared to the other camera types (p<0.001).

Spartan cameras of both models produced the highest empty image rates (fig. 1). However, the field team and image annotators reported 31 instances of SR3-CX cameras being knocked down for a prolonged period of time (>2 weeks), capturing many images of grass/sky not linked to camera performance. In comparison, only six such instances for Solar cameras (the next most knocked down camera type) were recorded. Adjusting for knockdown events would likely reduce Spartan SR3-CX empty image rates substantially.

All camera types showed noticeable unknown image proportions, except both Spartan models which exhibited minimal unknown captures.

The extensive significant differences for false triggers (wildlife vs empty images) partly reflects the large amount of data (~5 million images across 58,768 camera-days), which increases statistical power to detect even small effect sizes. This sensitivity is advantageous for robust camera performance evaluation.

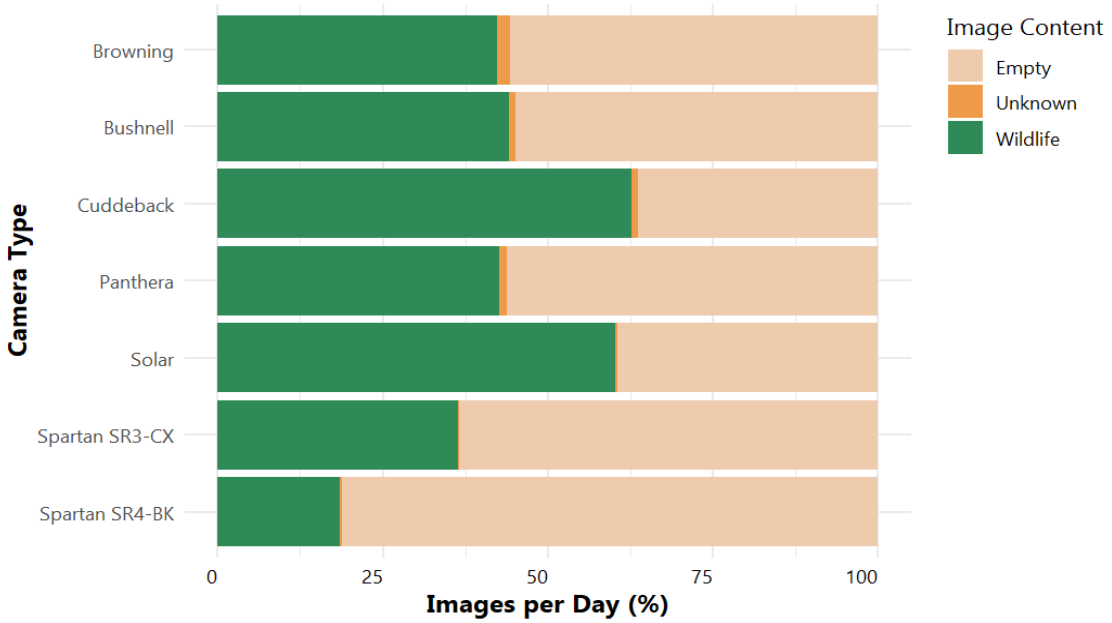


Fig. 1: Image content per day per camera type.

3.2 Sensitivity

In this overview, camera trap sensitivity refers to how easily a camera is triggered to take an image when motion is detected. In practice, sensitivity matters because cameras that trigger too often can generate many blank or low-value images, which drains batteries and in turn increases the need for field checks. However, cameras with low sensitivity may fail to detect wildlife, especially partially obscured animals, leading to missed detections. Understanding camera sensitivity is important when selecting equipment for different habitats and survey conditions. If cameras are deployed in tall grass, dense vegetation, a less sensitive camera may reduce false triggers and improve operational efficiency. In contrast, in more open habitats or when wildlife is sparse a more sensitive camera may be preferable to maximise capture probability.

To compare camera types, we calculated the number of images taken within each uninterrupted active period (N=863) for every camera deployed at a given location. Within an active period, the number of images taken by each camera was divided by the number of days on which that camera took images, producing an average image count per day for each camera, location, and active period. This approach allowed us to compare sensitivity across active periods, and thus different locations, rather than relying on a single aggregated value per camera type.

Before analysis, five extreme outliers were removed from the dataset where a camera exceeded an image-per-day value of 2,500 images. The resulting dataset, consisting of 858 active periods, was then used to assess differences among the remaining seven camera types (table 2). A Kruskal-Wallis test [6] showed highly significant differences in image-per-day values among camera types ($p<0.001$). Pairwise Wilcoxon rank-sum tests with Holm correction [4] indicated that Spartan SR4-BK was different from the other types with regards to sensitivity (fig. 2; table 3). When comparing two camera types, the type with the highest median value indicates the type with a higher sensitivity. The high median value for Spartan SR4-BK places it at the top of the sensitivity ranking (table 3). The remaining camera types showed a more moderate and partially overlapping pattern.

Table 2: The number of active periods that each camera type was deployed.

Camera Type	*Active periods*
Browning	27
Bushnell	77
Cuddeback	146
Panthera	54
Solar	81
Spartan SR3-CX	448
Spartan SR4-BK	25
	858

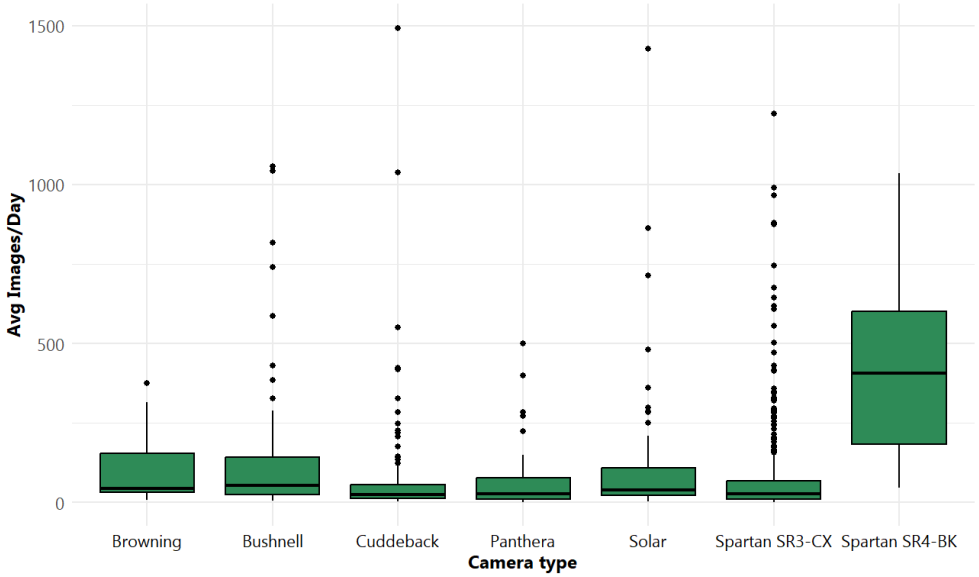


Fig. 2: A boxplot showing differences between camera types for the average number of images taken per day.

Table 3: Differences in camera type sensitivity based on a pairwise comparison using Wilcoxon rank sum test. Significance, values below 0.05, are indicated with asterisks with slightly significant (*; 0.01 – 0.05), intermediate (**; 0.001 – 0.01), and highly significant (***; <0.001).

	Browning	***Bushnell***	***Cuddeback***	***Panthera***	***Solar***	***Spartan SR3-CX***	**Median**
Browning	-	-	-	-	-	-	44.7
Bushnell	1.00	-	-	-	-	-	52.7
Cuddeback	0.04	<0.001***	-	-	-	-	23.9
Panthera	0.07	0.01*	1.00	-	-	-	27.0
Solar	1.00	1.00	0.01*	0.09	-	-	38.2
Spartan SR3-CX	0.06	<0.001***	1.00	1.00	0.02*	-	26.4
Spartan SR4-BK	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	406.0

3.3 Failure rate

Failure rate was assessed using two indicators: 1) the occurrence of software issues detected during deployment checks or while cameras were active, which could render images unreadable or prevent the camera from operating correctly, and 2) whether a camera reset itself to its factory default date after being knocked or disturbed, which complicates timestamp-based analyses. Breakage of camera units was not included in this metric, because physical damage is often strongly influenced by external conditions (e.g. presence of large animals, floodings or fires) rather than the reliability of the camera itself. For these analyses, a total of 1,456 active periods were included. This number is higher than that used for the sensitivity analysis because here we need less specific information (i.e. we do not need exact matches for first and last active days and number of days a camera was up).

The occurrence of software and resetting issues are shown in fig. 3. Bars are coloured green when the percentage of affected active periods remains below 5%, and red when this threshold is exceeded. This threshold is a project-specific benchmark and not a universal standard, because acceptable failure rates vary across computing and monitoring contexts. The software results show a clear gap between the green and red categories (fig. 3A). Browning had the lowest value among the red category, with 5.9% of active periods affected. In contrast, Spartan SR3-CX had the highest percentage of software issues among the camera types that remained in the green category, with 2.2% of active periods affected, which is less than half the ratio observed for Browning. Panthera showed the highest rate of software issues overall, with 8.5% of active periods affected by camera malfunction. A similar pattern was observed for date-resetting events (fig. 3B). Here, Panthera had the most resetting occurrences, with approximately 13.3% of active periods showing a camera that had reset to the default date during deployment. This ratio was more than three times higher than that of the next highest camera type, Spartan SR3-CX.

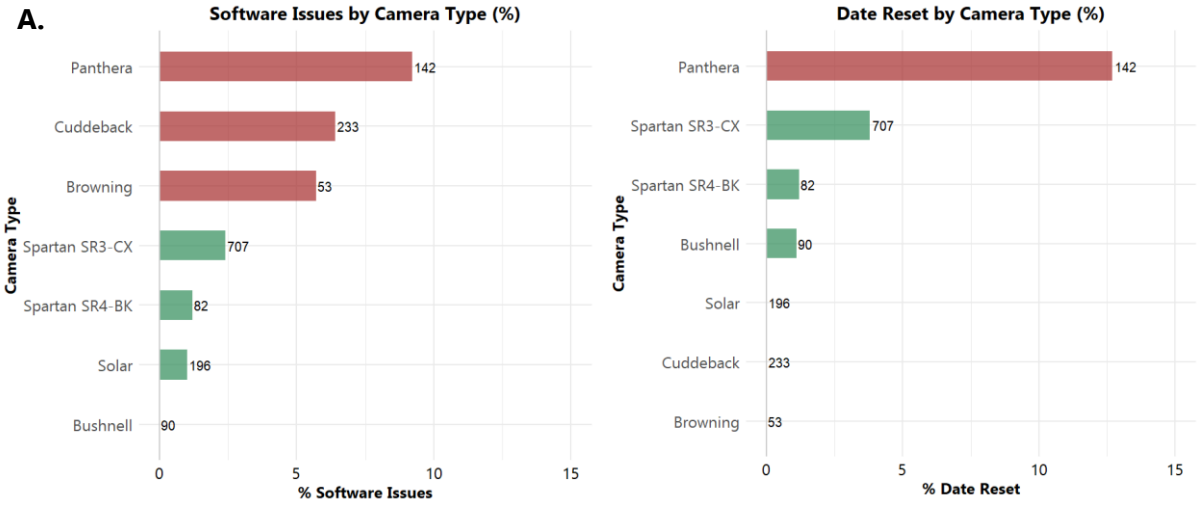


Fig.3: Camera type failure rate based on software issues (**A**) and date-resetting (**B**). The number on the right of each bar indicates the number of active periods represented for each camera type, while the x-axis shows the percentage of active periods in which software or resetting issues were recorded.

3.4 Camera performance

Camera performance was assessed by comparing the total duration of each active period with the number of days the camera was actually recording images. This provides a more complete overview of the outcomes captured in the sensitivity and failure-rate sections, as it combines several aspects of camera behaviour: battery drainage, physical damage, software-related interruptions, and missed triggers. A camera with strong performance should remain active for most of its deployment period and continue producing images consistently throughout that period. For this analysis, only active periods with camera checks no more than eight weeks apart were included. This restriction was applied because once checks are spaced beyond eight weeks, reduced performance is no longer attributable to the camera itself but instead likely reflects delayed maintenance.

Camera performance per type is shown in fig. 4. Bars are coloured red when performance falls below 66% (two-thirds). The figure shows a clear separation between the red camera types, Cuddeback and Panthera, both of which performed below 50%, and the green camera types. Although there is still ranking among the green types, the differences between them are relatively small, indicating broadly similar performance levels among the better-performing models. Overall, these results suggest that Cuddeback and Panthera were substantially less reliable in maintaining image capture across their active periods.

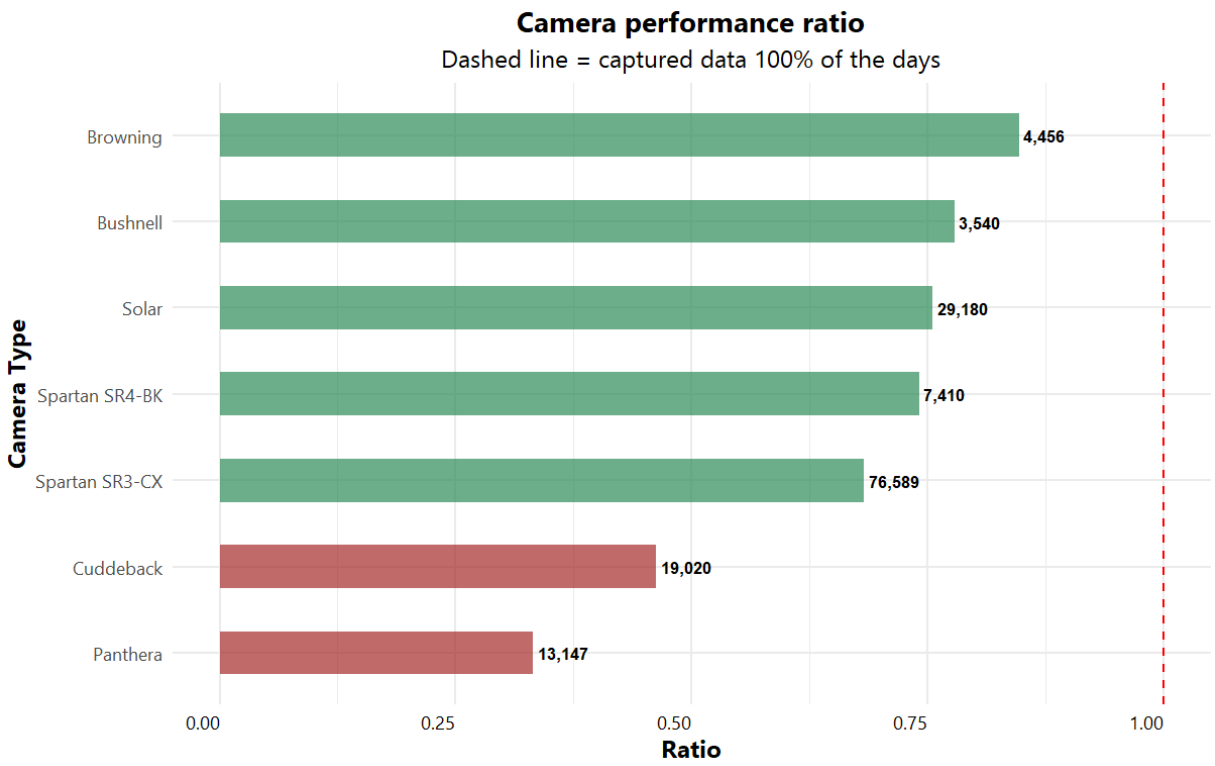


Fig.4: Camera type performance ratio. The number to the right of each bar represents the total length, in days, of all active periods combined for each camera type. The x-axis shows the proportion of these active period days during which the cameras took photos.

3.5 Image quality

Image annotators and field members noted several recurring camera-specific issues. Browning cameras were sometimes described as producing hazy night-time images, while Bushnell images showed a yellow shine that, at times, made them appear overexposed. Cuddeback cameras were reported to produce unusual colouration, including pink or yellow tones, or partially missing images. Cuddeback also required a larger number of batteries to run (eight instead of the six used in the other types). Panthera cameras were often described as difficult to get working, sometimes switching off after a day, not being compatible with all phones, and not showing dates on the images, which makes sorting and annotating more difficult. The panel of the solar cameras was considered a weak point in the event of animal encounters. Annotators also noted that Solar cameras can generate very large image volumes if not checked regularly due to almost no battery drainage. Browning, Bushnell and Solar only produce black-and-white night images. Spartan SR4-BK cameras were seen as incredibly sensitive, sometimes taking >10,000 images in a few days.

Each camera type also showed clear strengths. Solar cameras required very little battery replacement, making them especially suitable for long-term, low-maintenance monitoring. Both Spartan models were generally reliable in terms of image quality, with only a few instances of very poor or unusable images. Cuddeback and Spartan SR3 cameras demonstrated resilience under extreme conditions, having both survived fire events. Lastly, Browning, Bushnell, Panthera and Cuddeback cameras appeared relatively sturdy during animal encounters, with few breakages recorded.

3.6 Camera score-based performance

The results of species identifiability (wildlife capture and unknown images), software issues, date-reset issues, sensitivity (both too high and too low sensitivities gained a low score), operational performance, and image quality are combined in a ranking together with the affordability of each camera type (table 4). The affordability is based on the original full price, which ranges from 150 USD to 250 USD. This ranking suggests that Solar, Bushnell and the Spartan SR3-CX model performed best for this specific census. False triggers are not separately considered here, as it is basically a mirror image of the wildlife captures.

Table 4: Camera type ranking based on their suitability within the FRCC. For each category/column a score from 1 – 7 is applied, with 7 being the best performing type for that category. Note that this is project-specific and that the highest total sum shows the most suitable camera type for **this** census.

	Browning	***Bushnell***	***Cuddeback***	***Panthera***	***Solar***	***Spartan SR3-CX***	**Spartan SR4-BK**
Wildlife capture	3	5	7	4	6	2	1
Unknown images	1	2	4	3	5	7	6
Software failure	3	7	2	1	6	4	5
Date resetting	5	4	5	1	5	2	3
Sensitivity	3	2	2	4	4	3	1
Performance	7	6	2	1	5	3	4
Image quality	4	3	2	1	5	7	6
Affordability	1	5	4	2	3	1	1
	27	**34**	**28**	**17**	**39**	**29**	**27**

4 Conclusion

Taken together, the analyses show that camera type influences several important aspects of camera trap performance, but the differences are not always driven by a single metric alone.

The species identifiability and false-trigger results indicate that most camera types are relatively similar in their balance of empty, unknown, and wildlife images, although lower wildlife capture rates can also reflect cameras being knocked down, but continuing to take images while on the ground. Empty triggers are likewise not always caused by the camera itself, as rapid vegetation growth during spring and summer, or insufficient vegetation clearance during deployment, can increase the number of false triggers.

The sensitivity analysis demonstrated that camera type has a strong effect on daily image output. Spartan SR4-BK emerged as the most sensitive camera type, while the other models clustered into intermediate or lower sensitivity categories. Likely because of this high sensitivity, this specific model also had the most false triggers. High sensitivity is not inherently negative, as it can be beneficial in open landscapes or for monitoring species that are small, elusive, or rapidly moving. However, in this census, a highly sensitive camera type drains batteries quickly, and the large-scale of the census did not allow for easy maintenance.

The software and date-reset analyses showed that Panthera is particularly vulnerable to both software-related problems and timestamp failures after disturbance. These results highlight that camera selection for long-term monitoring must consider not only detection performance, but also reliability, maintenance demand, and data usability.

Selecting the right camera for the research question is critical as no single camera type performs best in every setting. The best choice depends on the habitat, target species, maintenance schedule, and the balance between detection probability, battery use, and data quality. For the FRCC this meant that, because cameras cannot be checked daily or even weekly due to the vastness of the study area, they must remain functional for prolonged periods of time, across seasons and under extreme temperature and weather conditions. Overall, the combined ranking suggests that Solar, Bushnell and the Spartan SR3-CX performed best in this specific context.

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Appendix VI

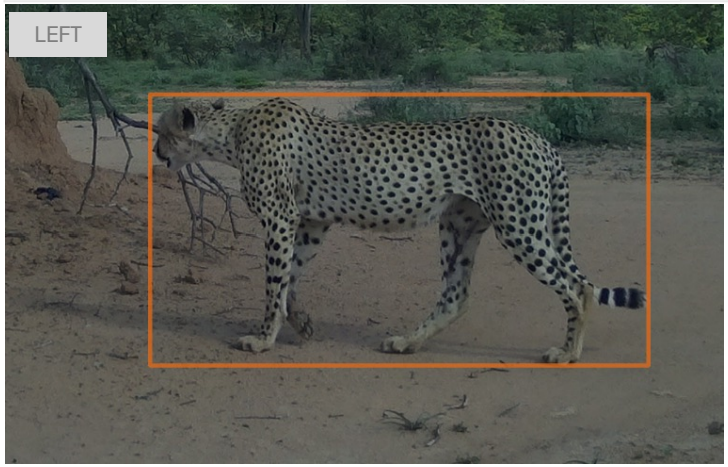
Free-Roaming Cheetah Census

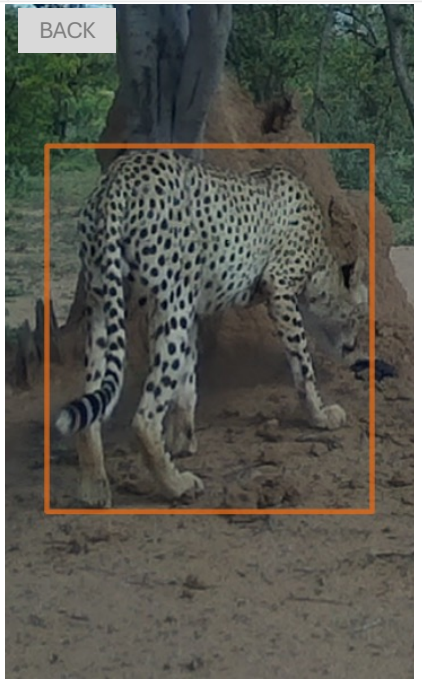
INDIVIDUAL IDENTIFICATION KIT

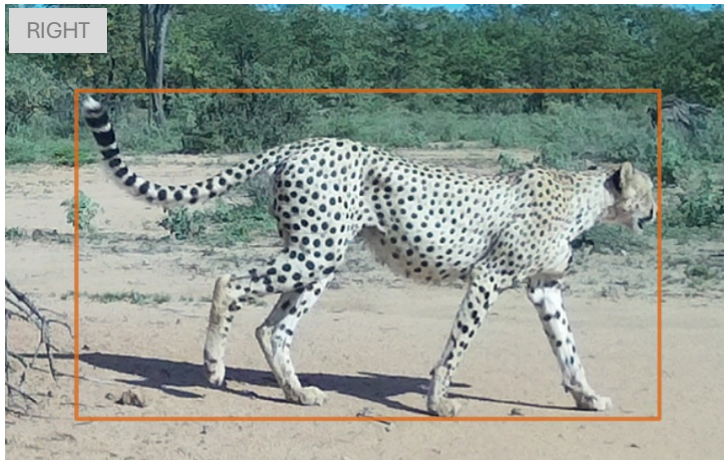
FRCC_013

Acinonyx jubatus jubatus | Southern African cheetah

				LK status	Alive	Maternal lineage	Name	Location	Paternal lineage	Name	Location
				Sex	Male	Dam:	*UNKNOWN*	*UNKNOWN*	Sire	*UNKNOWN*	*UNKNOWN*
Date first identified	14-09-2023	Date last identified	18-05-2025	Est. DOB	NA	Dam mother:	*UNKNOWN*	*UNKNOWN*	Sire mother	*UNKNOWN*	*UNKNOWN*
Detection method	Camera trap	Identified by:	Nynke Wemer	LK age class	Adult	Dam father:	*UNKNOWN*	*UNKNOWN*	Sire father	*UNKNOWN*	*UNKNOWN*
Grid:	LP_M2, LP_L2	Location (lat):	-22,4547	Location (long):	29,2587	# Images	635	Confidence (1-5)	5		
Microchip:	NA	Collar:	NA	Coalition:	FRCC_014					Place of ID	TrapTagger







NOTES

Site LP_M2_002
Site LP_M2_008
Site LP_M2_009
Site LP_M2_011
Site LP_M2_012
Site LP_L2_005

Appendix VI

Free-Roaming Cheetah Census

INDIVIDUAL IDENTIFICATION KIT

FRCC_015

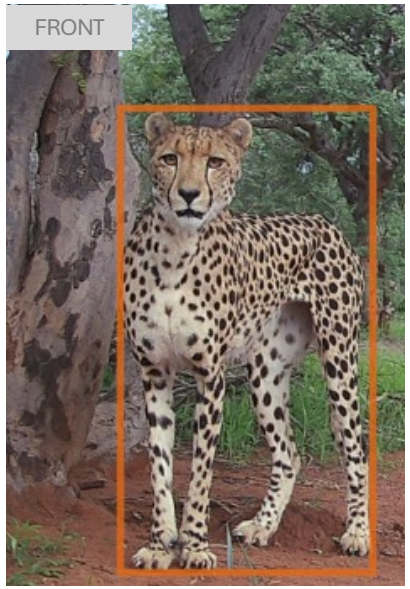
Acinonyx jubatus jubatus | Southern African cheetah

				LK status	Alive	Maternal lineage	Name	Location	Paternal lineage	Name	Location
				Sex	Female	Dam:	*UNKNOWN*	*UNKNOWN*	Sire	*UNKNOWN*	*UNKNOWN*
Date first identified	12-02-2023	Date last identified	27-12-2024	Est. DOB	NA	Dam mother:	*UNKNOWN*	*UNKNOWN*	Sire mother	*UNKNOWN*	*UNKNOWN*
Detection method	Camera trap	Identified by:	Nynke Wemer	LK age class	Adult	Dam father:	*UNKNOWN*	*UNKNOWN*	Sire father	*UNKNOWN*	*UNKNOWN*
Grid:	LP_I4, LP_J4	Location (lat):	-22,8634	Location (long):	28,4056	# Images	52	Confidence (1-5)	5		
Microchip:	NA	Collar:	NA	Coalition:						Place of ID	Farmer & TrapTagger

LEFT

RIGHT

FRONT



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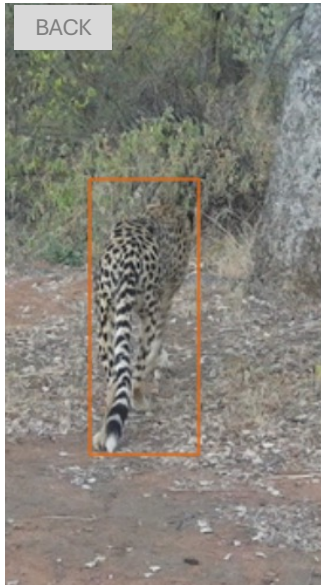


NOTES

Site LP_I4_001
Site LP_I4_004

Also captured by Chanelle Becker at Zingela, LP_J4 (small images) Oct 14 2024
Seen together there with FRCC_077 and FRCC_078

Appendix VI

Free-Roaming Cheetah Census

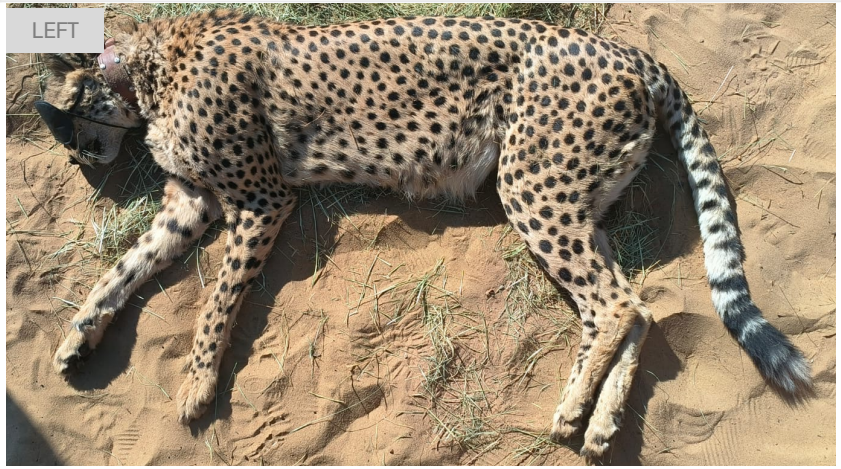
INDIVIDUAL IDENTIFICATION KIT

FRCC_044

Acinonyx jubatus jubatus | Southern African cheetah

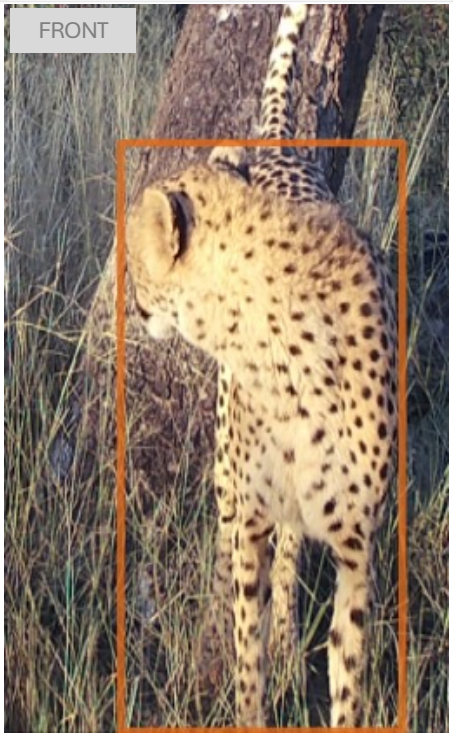
					LK status	Deceased	Maternal lineage	Name	Location	Paternal lineage	Name	Location
					Sex	Female	Dam:	*UNKNOWN*	*UNKNOWN*	Sire	*UNKNOWN*	*UNKNOWN*
Date first identified	07-12-2023	Date last identified	01-06-2025		Est. DOB	NA	Dam mother:	*UNKNOWN*	*UNKNOWN*	Sire mother	*UNKNOWN*	*UNKNOWN*
Detection method	Camera trap	Identified by:	Nynke Wemer		LK age class	Adult	Dam father:	*UNKNOWN*	*UNKNOWN*	Sire father	*UNKNOWN*	*UNKNOWN*
Grid:	LP_F7, LP_E7	Location (lat):	-23,5771	Location (long):	27,5479	# Images	80	Confidence (1-5)	5			
Microchip:	NA	Collar:	ST1269	Coalition:							Place of ID	TrapTagger

LEFT

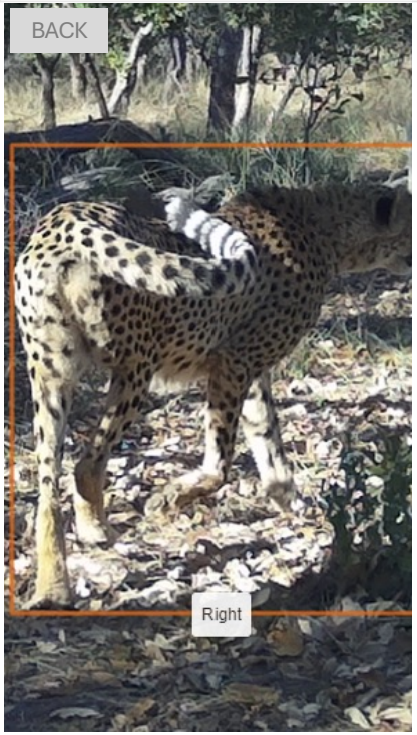


RIGHT

FRONT



BACK



NOTES

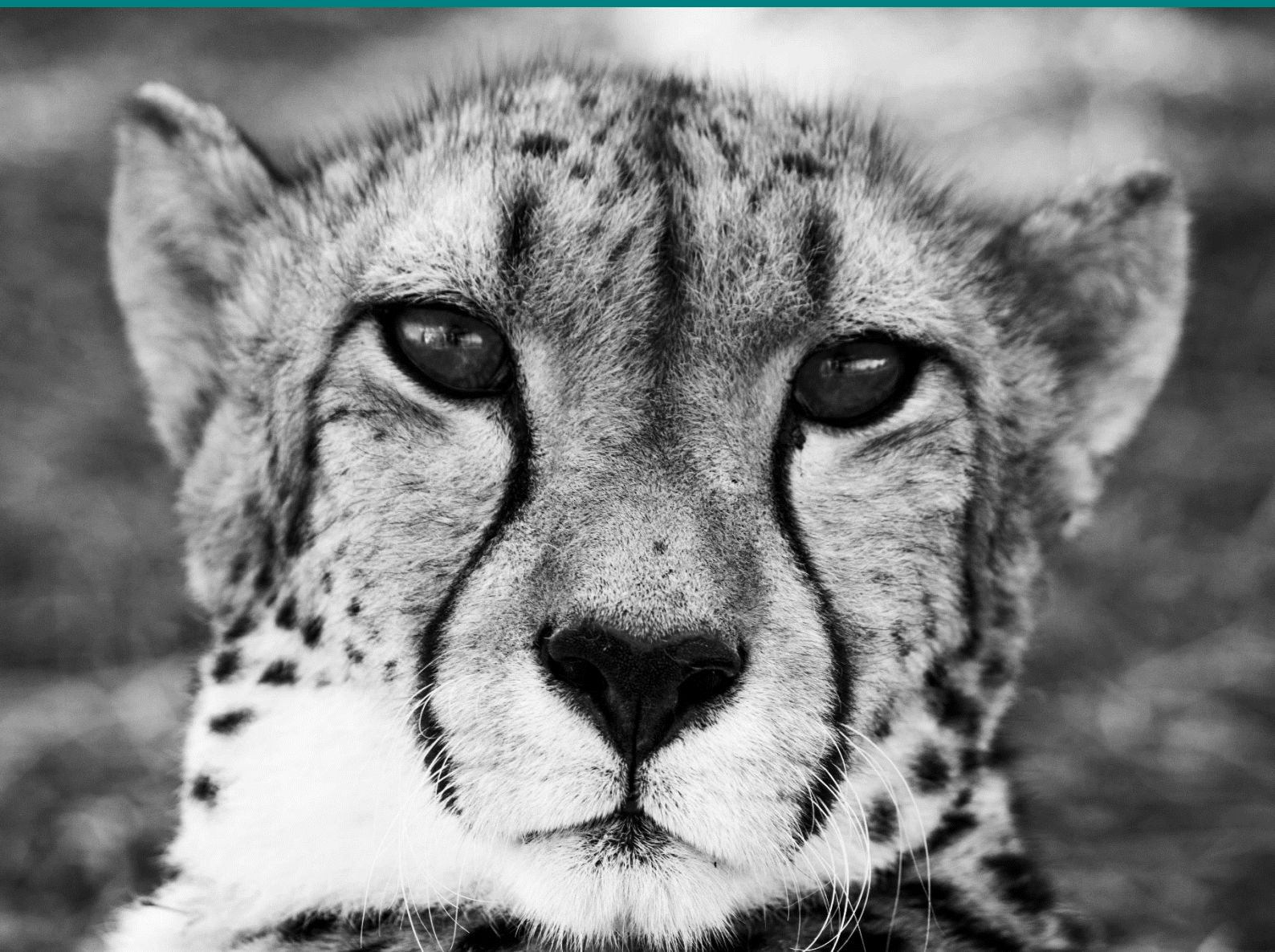
Site LP_F7_001
Site LP_F7_002

Site LP_E7_001
Manketti area

Died on 4th of June 2025, reason unknown

Medical Laboratories
Human and Animal Genetic Testing

Assessing Genetic Diversity and Population Structure of the Free-Roaming Cheetah Populations of South Africa Using Short Tandem Repeat (STR) Markers



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1. Introduction

The African cheetah (*Acinonyx jubatus jubatus*) is classified as vulnerable due to habitat loss, human-wildlife conflict, and reduced genetic diversity. Understanding the genetic structure and diversity within this population is crucial for effective conservation strategies. This project aims to assess the genetic diversity within groups of the African cheetah population by utilizing Short Tandem Repeat (STR) markers to determine key genetic parameters such as heterozygosity, Shannon's Information Index, polymorphic information content, fixation index, Hardy-Weinberg equilibrium, Nei's genetic distance, inbreeding, relatedness, and subpopulation assignment.

2. Materials and Methods

2.1 Sample Collection and DNA Extraction

- Sample collection: blood spots on Guthrie cards, whole blood samples (in purple top EDTA tubes) and tissue samples were collected from free-roaming cheetahs in South Africa.
- Genomic DNA was extracted using standardized protocols to ensure high-quality genetic material.

2.2 STR Marker Genotyping

- Genomic DNA was amplified using polymerase chain reaction (PCR) and genotyping performed through capillary electrophoresis.

Table 1: 18 STR markers amplified during polymerase chain reaction (PCR) for cheetah samples.

Marker Name	Forward and Reverse Sequence	Size Range	Dye
FCA001	F: TGC TTG TCC TCT CCC TCG R: TGA CTG CGC CAT AGC TTT C	130 – 150	NED
FCA026	F: GGA GCC CTT AGA GTC ATG CA R: TGT ACA CGC ACC AAA AAC AA	115 – 160	FAM
FCA031	F: GCC AGG GAC CTT TAG TTA GAT T R: GCC CTT GGA ACT ATT AAA ACC A	220 – 240	FAM
FCA042	F: CCC ACG TGG ACT AAT CAA AT R: CAC TGC ACA AAT TAA GAG GC	200 – 240	PET
FCA057	F: AAG TGT GGG ATT GGG TGA AA R: CCA TAA GAG GCT CTT AAA AAC TGA	140 – 160	VIC
FCA069	F: AAT CAC TCA TGC ACG AAT GC R: AAT TTA ACG TTA GGC TTT TTG CC	80 – 110	VIC
FCA075	F: ATG CTA ATC AGT GGC ATT TGG R: GAA CAA AAA TTC CAG ACG TGC	110 – 140	NED
FCA097	F: TAA TGT TCA ACT TGA ATT GCT TCC R: GAA CAG TAG TTT GCC CAT ACA GG	130 – 160	VIC
FCA105	F: TTG ACC CTC ATA CCT TCT TTG G R: TGG GAG AAT AAA TTT GCA AAG C	180 – 210	PET
FCA113	F: TGG GAC GTC ATT GCT CCT R: CCC ACA TTG GGC ATA GAA AT	140 – 160	VIC
FCA126	F: AAG GCA ACA TGG CCC CTG AT R: ACT CTA GCA CAG AGC CAA GAG C	180 – 200	PET
FCA224	F: CTG GGT GCT GAC AGC ATA GA R: TGC CAG AGT TGT ATG AAA GGG	150 – 170	FAM

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FCA240	F: TCT TTA AGA TGG CCG GAC TG R: TCC CCT CAA ATA TGC AAA GG	170 – 180	FAM
FCA272	F: ACC TTT ACC TCC TTC CAA AAA G R: CAC CTT TCC ATC CAA TAA ATT C	100 – 130	FAM
FCA310	F: TTA ATT GTA TCC CAA GTG GTC A R: TAA TGC TGC AAT GTA GGG CA	120 – 150	FAM
FCA391	F: GCC TTC TAA CTT CCT TGC AGA R: TTT AGG TAG CCC ATT TTC ATC A	200 – 230	NED
FCA453	F: AAT TCT GAG AAC AAG CTG AGG G R: ATC CTC TAT GGC AGG ACT TTG	180 – 200	PET
FCA506	F: AAT GAC ACC AAG CTG TTG TCC R: AGA ATG TTC TCT CCG CGT GT	230 – 250	NED

2.3 Genetic Diversity Statistics Calculation

To support clear interpretation and application of the genetic analyses, Table 2 provides an overview of the diversity indices used in this study, including their purpose, interpretation, and the expected values indicative of favourable population genetic outcomes.

Table 2: Summary of genetic diversity indices used in this study, outlining the purpose of each metric, guidance on interpretation, typical value ranges, and outcomes indicative of favourable population genetic status.

Diversity indices	Purpose	Interpretation	Value	Favourable Outcomes
Sub-population Assignment *STRUCTURE & GENALEX*	Analyses how genetically similar samples are to each other and groups them into sub-populations accordingly.	• Animals that cluster together likely breed more with each other. • Animals showing mixed membership ("admixture") likely move and interbreed between areas.	Allows us to understand whether there is gene flow between groups of animals (random mating structure) or whether some animals/groups are becoming genetically isolated.	• **Free-roaming populations:** Some structure is normal, but mixing between groups is healthy. • **Cheetahs:** Mild structure is expected, but strong separation can signal habitat fragmentation or fencing effects.
Number of Alleles (N_a) *GENALEX*	Calculates how many variants are found at each genetic marker within the dataset.	• **Higher N_a (2 – 5):** indicates a greater variety of genetic options within the group. • **Lower N_a (1.5 – 2):** suggests the group has a limited number of alleles, which can often be attributed to previous population declines, small population size and isolation or limited gene flow.	• Identifies uncommon alleles that might not significantly influence heterozygosity. • Aids in monitoring the decline of genetic diversity over time. • Valuable for pinpointing populations that may have experienced bottlenecks. • Crucial for assessing long-	• **Free-roaming populations:** a higher number of alleles per locus is generally favourable, with values above 4 -6 indicating good genetic diversity, while very low values (<2) may suggest reduced variation or isolation. • **For cheetahs:** lower allele numbers are expected due to historical

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		• **N_a values lower than 1.5:** can be seen as a cause for concern.	term adaptive capacity, even when the present health of the population appears stable.	population bottlenecks, and values of 2 - 5 alleles per locus are considered normal and stable, with concern only arising when most loci show fewer than ~1.5 alleles.
Effective Number of Alleles (N_e) *GENALEX*	This estimates how many alleles are effectively contributing to genetic diversity, taking into account how evenly allele frequencies are distributed.	• **If N_e value is close to N_a:** alleles are evenly distributed. • **If N_e value is much lower than N_a:** a few alleles dominate the population. • **N_e of 1.3 – 2.5:** adequate diversity. • **N_e of 1.1 – 1.3:** caution is advised, monitor population. • **Very low N_e (<1.1):** cause for concern - reduced functional diversity, even if multiple alleles exist. • N_e is therefore more informative than N_a for understanding real genetic diversity.	• Reflects usable genetic diversity. • Less sensitive to rare alleles and sample size than N_a. • Strong indicator of: population stability, genetic drift and long-term evolutionary potential.	• **Healthy cheetah pattern:** both N_a and N_e is low but values remain stable across loci and groups over time. • **Early warning pattern:** • N_a moderately high but N_e is very low – this would suggest that some alleles although present, are being lost through genetic drift.
Shannon's Information Index (S_I) *GENALEX*	This statistic measures how many different genetic variants exist, how common they are and how evenly their presence is distributed through the dataset.	• **Greater values (0.4 – 1.0):** indicate increased genetic diversity, with numerous variants distributed more uniformly across the population. • **Smaller values (<0.39):** indicates a limited range of options, or the population might consist of	Populations with more evenly distributed genetic variants tend to be more stable.	• **Free-roaming populations:** Higher values indicate more diversity. • **Cheetahs:** Lower values are expected, but very low values may signal further diversity loss.

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		numerous individuals that possess only a small number of shared variants. • Should S_I decreases over time (or is significantly lower in one region compared to another), it may indicate a reduction in diversity, isolation, or a limited effective breeding population.		
Heterozygosity (H_o and H_e) *MSATTOOLS*	This statistic is utilised to assess the degree of genetic diversity present within a population. **H_e:** what we expect to see. **H_o:** What we observe.	• **H_o and H_e are close in value:** indicates healthy genetic mixing. • **H_o lower than H_e:** Possible inbreeding or separation into sub-groups. • **Values between 0.3 – 0.5:** no cause for concern. • **Values below 0.3:** caution. • **Values below 0.25:** concerning.	Higher genetic variation generally indicates better adaptability and resilience to diseases and environmental changes.	Within **free-roaming populations**, higher values are considered more favourable. However, within **cheetah groups**, values are expected to be lower than in many species. Comparison thereof should therefore be relative and not absolute.
Polymorphic Information Content (PIC) *MSATTOOLS*	This statistic does not describe the population itself, but rather measures how useful each genetic marker is within the dataset.	• **Values above 0.5:** excellent informativeness. • **Values between 0.3 – 0.55**: acceptable for cheetah populations. • **Values below 0.3:** low informativeness.	Using informative markers improves confidence in other analyses.	• Higher PIC values translate to higher marker informativeness. • Moderate values are acceptable for cheetahs due to their reduced genetic diversity.
Fixation Index (F_{IS}) *GENALEX* and *FSTAT*	This statistic measures whether animals within the group are more genetically similar than would be expected. This indicates whether animals within a sub-population is	• **Values close to 0 (0 - 0.10):** indicates that individuals within the group are likely mating at random (showing no significant signs of inbreeding).	Positive values may indicate inbreeding, restricted mate choice or hidden population subdivision.	• **Free-roaming populations:** Values close to 0 are ideal. • **Cheetahs:** Slightly higher values are common, but consistently high

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	breeding mostly among themselves.	• **A higher positive F_{IS} value (0.1 – 0.15):** suggests there is less diversity observed within the group than expected, which can occur due to inbreeding or hidden substructures within the dataset. Values greater than 0.15 should be seen as a cause for concern. • **A negative F_{IS} value:** indicates more diversity within the group than expected, which may arise from admixture between groups or sampling bias within the dataset. • Should positive F_{IS} value be consistently observed, it may indicate limited breeding options between animals or breeding isolation between groups.		values may be cause for concern.
Hardy-Weinberg Equilibrium (HWE) *GENEPOP*	Markers adhering to HWE indicates whether a population behaves as would be expected if random mating is occurring and no strong selection or isolation drives are in effect.	• For random mating populations, we expect markers to adhere to HWE. • Should a marker deviate from HWE, it may be due to non-random mating or inbreeding, population subdivision (samples contain more than one breeding group), or selection drive.	When many genetic markers deviate from this expectation, it can indicate: • Inbreeding • Population structure; • or recent demographic changes	• Occasional deviations are normal. • Widespread deviations suggest non-random mating or population subdivision.

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Inbreeding coefficient *COANCESTRY*	This statistic estimates how likely it is that an animal inherited the same genetic material from both parents, i.e., how closely related were the animal's parents to each other.	• **Values close to 0 (0 – 0.1):** parents were not closely related. • **Higher values (0.1 – 0.15):** increased level of inbreeding. Values larger than 0.15 should be seen as cause for concern.	High inbreeding can reduce fertility, survival, and disease resistance over time.	• **Free-roaming populations:** Low values are best. • **Cheetahs:** While some inbreeding is anticipated as a result of historical population declines, excessively high levels could be concerning, particularly if they continue to rise.
Relatedness *COANCESTRY*	This statistic measures how genetically similar two animals are. It estimates whether two animals are likely to be close relatives or not.	• **High values (0.1 – 0.2):** likely close family (siblings, parent-offspring). Values larger than 0.2 can be seen as cause for concern. • **Values close to 0 (-0.05 to 0.1):** Unrelated animals.	If a large number of animals within a group share close genetic ties, it may indicate: • Restricted movement of animals. • Limited options for mating. • Reduced population size.	• **Free-roaming populations:** The majority of animals should not be closely related. • **Cheetahs:** A certain level of relatedness is typical, but a population primarily composed of close relatives could face potential risks.

3. Results and Discussion

52 samples were received from Ashia farm. DNA was extracted from whole blood, blood on Guthrie cards and tissue using the E.Z.N.A. Blood DNA Mini Kit. 39 samples were successfully amplified on the UML Feline Predator STR panel. Genders (Table 3) were assigned to samples for which the X/Y STR marker amplified successfully:

Table 3: Assigned sample genders as determined by the X/Y STR marker pair.

Lab number	Tag	Microchips	Gender
U60522	FRP0001 (88448)	985113000799191	Male
U60523	FRP0002 (88469)	992003000154259	Female
U60525	FRP0004 (88527)	992993999156494	Female
U60526	FRP0005 (88525)		Male
U60528	FRP0007 (72576)	945000001635354	Female
U60529	FRP0008 (72613)	900182001425107	Male
U60530	FRP0009 (72620)		Male
U60531	FRP0010 (72638)	0006433A36	Male
U60534	FRP0013 (72710)	0006341F46	*Undetermined*
U60535	FRP0014 (72729)	000689232F	*Undetermined*
U60536	FRP0015 (72733)		Male
U60537	FRP0016 (72734)	42321D0357	*Undetermined*
U60539	FRP0018 (72739)	477E536815	Male
U60540	FRP0019 (72741)	43104B404B	*Undetermined*

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U60542	FRP0021 (104950)	OCC600025383	Male
U60543	FRP0022 (104949)	992003000156490	Female
U60544	FRP0023 (104951)	992003000156493	Female
U60545	FRP0024 (104952)		Male
U60546	FRP0025 (72732)		Male
U60547	FRP0026 (72738)	476D7E7806	Male
U60549	FRP0028 (88640)	4C45042D70	Male
U60552	FRP0031 (87588)	953010004662895	Male
U60554	FRP0033 (86491)	953010004662912	Female
U60555	FRP0034 (86490)	953010004662916	Male
U60558	FRP0037	4864063F11	*Undetermined*
U60560	FRP0039 (86487)	953010004662900	Male
U60561	FRP0041 (88639)		Male
U60562	FRP0042 (72730)	486A0F0920	Male
U60563	FRP0043	45A390C33	Male
U60564	FRP0044 (72753)		*Undetermined*
U60565	ACC001	992003000382401	*Undetermined*
U60568	ACC011	4C4605346C	Female
U60569	ACC013	900141000234580	Female
U60570	ACC015	900141000234575	Female
U60572	ACC020	900141000066327	*Undetermined*
U60573	ACC021	941000026459902	*Undetermined*
U60575	ACC026	992003000382404	*Undetermined*
U60576	ACC028	900141000898519	Male
U60577	ACC029	900141000898517	Female
U60578	ACC030	900141000898518	Female
U60579	ACC031	900141000898514	Male
U60580	ACC032	900141000898511	Female
U60581	ACC033		Female
U60654	900182002321430	900182002321430	Male
U60655	993021200013922	993021200013922	Male
U60656	FR CC CHEETAH SKIN		Male

3.1 Diversity Indices Statistics:

Population statistics were run on the generated STR profiles. Datasets were divided into samples originating from Group A (Limpopo and Botswana) and Group B (North West and Northern Cape) (see Tables 4 – 5; Figures 1 - 3). Number of alleles (N_a) ranged from 2 to 7 for both Group A and Group B samples. Effective number of alleles (N_e) ranged from 1.5 to 4.94 for Group A samples and from 1.22 to 5.04 for Group B samples. Shannon's Information Index (S_I) ranged from 0.52 to 1.76 for Group A samples and 0.33 to 1.73 for Group B samples.

Observed heterozygosity (H_o) ranged from 0.09 to 0.82 for Group A samples and 0.20 to 0.91 for Group B samples, while expected (H_e) heterozygosity ranged from 0.34 to 0.82 for Group A samples and 0.18 to 0.80 for Group B samples. Polymorphic Information content ranged from 0.28 to 0.77 for Group A samples and 0.16 to 0.77 for Group B samples. Fixation Index (F_{IS}) ranged from -0.18 to 0.88 for Group A samples and from -0.33 to 0.63 for Group B samples. For Group A, 5 markers were shown to violate Hardy-Weinberg Equilibrium, while 6 markers violated HWE for Group B samples.

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*Table 4: Number of alleles (N_a), number of effective alleles (N_e), Shannon's information index (S_I), observed heterozygosity (H_o), expected (H_e), polymorphic information content (PIC), fixation index (F_{IS}) and Hardy-Weinberg equilibrium (HWE) across the 18 STR markers for Group A dataset. HWE can be interpreted as ns = not significant, marker in HWE; * = significant, marker violates HWE.*

	Na	Ne	SI	Ho	He	PIC	FIS	HWE
FCA001	2	1.86	0.655	0.545	0.474	0.356	-0.179	ns
FCA026	6	4.52	1.595	0.636	0.797	0.744	0.183	ns
FCA031	5	2.65	1.205	0.455	0.637	0.573	0.270	ns
FCA042	6	4.40	1.568	0.682	0.791	0.735	0.118	ns
FCA057	5	2.85	1.225	0.636	0.664	0.586	0.019	ns
FCA069	5	3.13	1.314	0.455	0.697	0.630	0.332	ns
FCA075	6	4.23	1.546	0.409	0.781	0.725	0.464	*
FCA097	5	2.00	1.018	0.545	0.513	0.469	-0.089	ns
FCA105	4	3.72	1.351	0.545	0.748	0.683	0.254	*
FCA113	5	3.78	1.464	0.682	0.753	0.697	0.073	ns
FCA126	5	3.34	1.331	0.636	0.717	0.647	0.091	ns
FCA224	7	3.13	1.375	0.591	0.697	0.627	0.132	*
FCA240	5	4.50	1.556	0.091	0.796	0.743	0.883	*
FCA272	4	3.08	1.208	0.667	0.692	0.614	0.013	ns
FCA310	5	3.32	1.301	0.818	0.715	0.641	-0.172	ns
FCA391	6	4.59	1.614	0.714	0.801	0.749	0.087	ns
FCA453	2	1.50	0.515	0.316	0.341	0.277	0.050	ns
FCA506	7	4.94	1.758	0.591	0.816	0.772	0.259	*
Average	4.8	3.29	1.242	0.527	0.639	0.626	0.155	N.A

*Table 5: Number of alleles (N_a), number of effective alleles (N_e), Shannon's information index (S_I), observed heterozygosity (H_o), expected (H_e), polymorphic information content (PIC), fixation index (F_{IS}) and Hardy-Weinberg equilibrium (HWE) across the 18 STR markers for the Group B dataset. HWE can be interpreted as ns = not significant, marker in HWE; * = significant, marker violates HWE.*

	Na	Ne	SI	Ho	He	PIC	FIS	HWE
FCA001	2	1.862	0.655	0.545	0.463	0.3557	-0.179	ns
FCA026	5	3.025	1.275	0.727	0.669	0.6134	-0.086	*
FCA031	3	1.449	0.576	0.364	0.310	0.2822	-0.173	ns
FCA042	7	5.042	1.732	0.818	0.802	0.7727	-0.021	*
FCA057	4	2.602	1.118	0.818	0.616	0.5603	-0.329	ns
FCA069	5	2.241	1.112	0.545	0.554	0.5192	0.015	*
FCA075	5	4.400	1.535	1.000	0.773	0.7352	-0.294	ns
FCA097	4	2.547	1.073	0.545	0.607	0.5334	0.102	ns
FCA105	4	2.142	0.983	0.455	0.533	0.4818	0.147	ns
FCA113	4	2.068	0.923	0.455	0.517	0.4523	0.120	*
FCA126	3	2.142	0.837	0.545	0.533	0.4316	-0.023	ns
FCA224	4	2.373	0.993	0.727	0.579	0.4914	-0.257	*
FCA240	4	3.841	1.365	0.273	0.740	0.6914	0.631	*
FCA272	5	3.103	1.286	0.636	0.678	0.6196	0.061	ns
FCA310	5	3.143	1.304	0.909	0.682	0.6299	-0.333	ns
FCA391	5	3.967	1.475	0.727	0.748	0.7061	0.028	ns
FCA453	2	1.220	0.325	0.200	0.180	0.1638	-0.111	ns
FCA506	7	5.042	1.732	0.727	0.802	0.7727	0.093	ns
Average	4.16	2.8	1.068	0.580	0.568	0.545	-0.034	N. A

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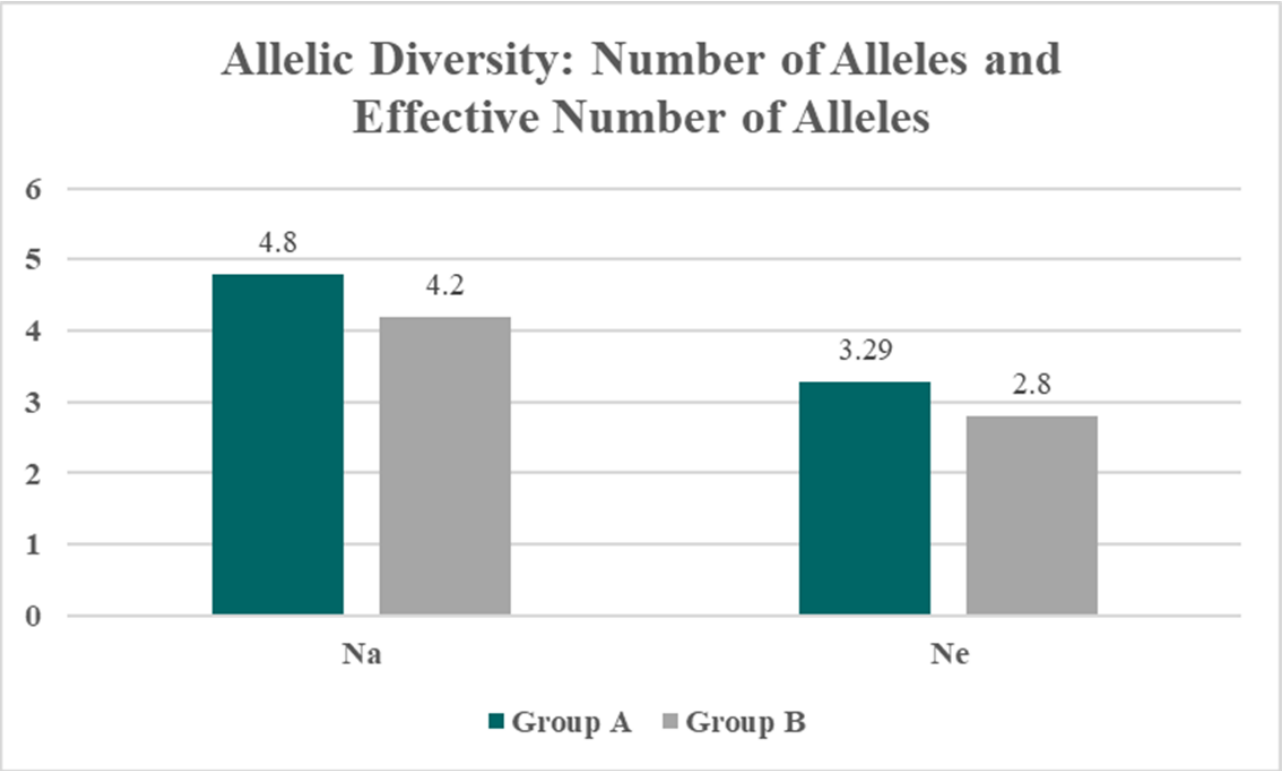

Figure 1: Comparison of allelic diversity across the Group A and Group B datasets as calculated in GenAlEx. N_a: Number of alleles; N_e: Number of Effective alleles.

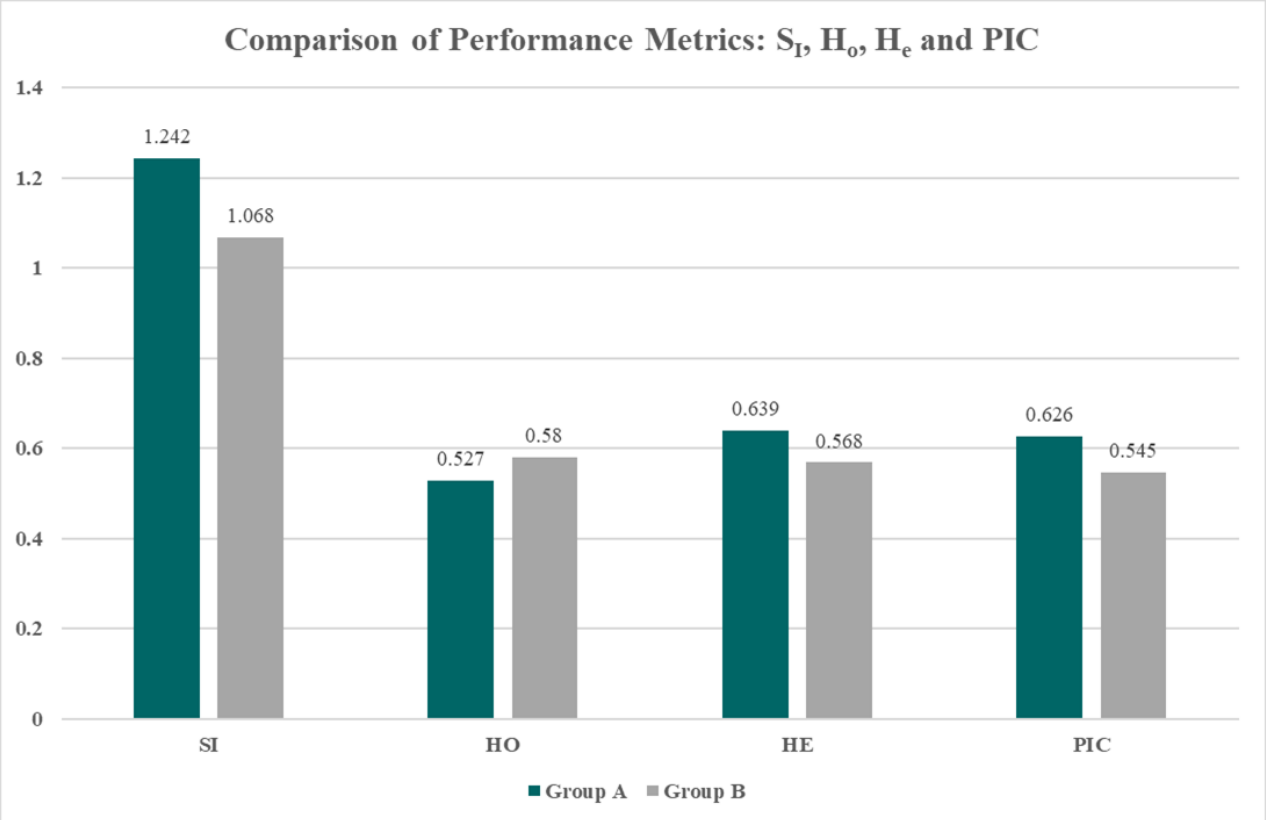

Figure 2: Comparison of performance metrics across the Group A and Group B datasets as calculated in GenAlEx. S_I: Shannon's Information Index; H_o: Observed Heterozygosity; H_e: Expected Heterozygosity; PIC: Polymorphic Information Content.

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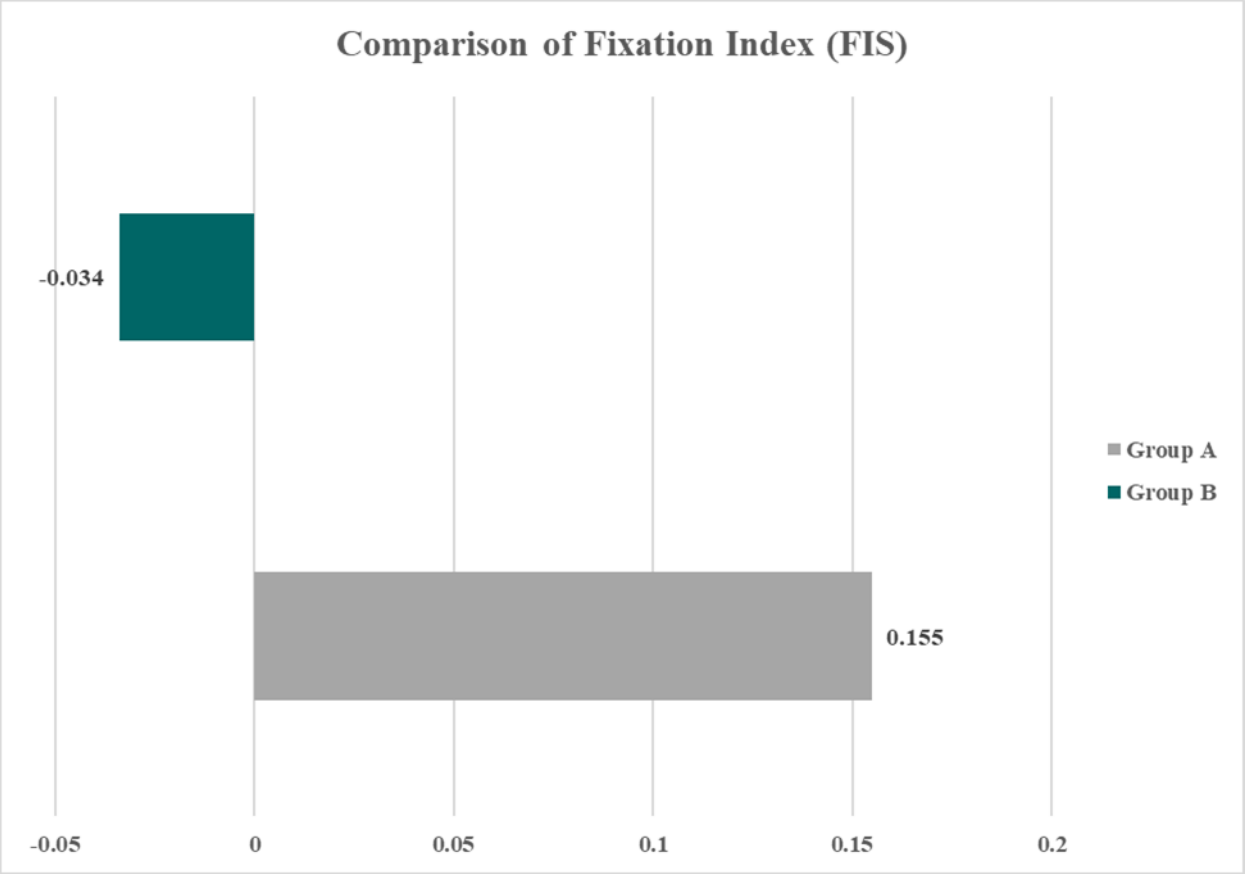

Figure 3: Comparison of Fixation Index (F_{IS}) across the Group A and Group B datasets as calculated in GenAlEx.

3.2 Interpretation of Genetic Diversity Indicators:

Group A dataset

The Group A dataset exhibited moderate allelic diversity, with a mean number of alleles per locus (N_a = 4.8) and an effective number of alleles (N_e = 3.3), indicating a relatively even distribution of alleles across loci. The Shannon information index (S_I = 1.24) further supports the presence of moderate genetic diversity within this group. Observed heterozygosity (H_o = 0.53) was lower than expected heterozygosity (H_e = 0.64), resulting in a positive inbreeding coefficient (F_{IS} = 0.155). This pattern indicates a deficit of heterozygotes, suggesting possible inbreeding, population substructure, or the presence of related individuals within the sampled population. The mean polymorphic information content (PIC = 0.63) indicates that the markers are highly informative and suitable for assessing genetic diversity and population structure. While most loci conformed to Hardy–Weinberg expectations, five loci showed significant deviations, which may reflect localized substructure, small population effects, or sampling of related individuals rather than widespread genetic instability.

Group B dataset

The Group B dataset exhibited moderate allelic diversity, with a mean number of alleles per locus (N_a = 4.16) and an effective number of alleles (N_e = 2.80), indicating a reasonably even distribution of alleles across loci. The Shannon information index (S_I = 1.07) further supports the presence of moderate genetic diversity within this group. Observed heterozygosity (H_o = 0.580) slightly exceeded expected heterozygosity (H_e = 0.568), resulting in a mildly negative inbreeding coefficient (F_{IS} = −0.034). This pattern suggests a small excess of heterozygotes and indicates little evidence of

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widespread inbreeding within the population, with heterozygosity levels broadly consistent with Hardy-Weinberg expectations overall.

The mean polymorphic information content (PIC = 0.545) indicates that the markers remain moderately to highly informative and suitable for assessing genetic diversity and population structure within the dataset. Several loci, including FCA042 and FCA506, displayed particularly high PIC values, reflecting strong discriminatory power at these markers. Although most loci conformed to Hardy-Weinberg equilibrium, several loci showed significant deviations, which may reflect localized relatedness, stochastic allele-frequency variation, or fine-scale substructure rather than widespread genetic instability. Overall, the Group B dataset reflects a relatively well-mixed and genetically stable population with moderate diversity and limited evidence of extensive inbreeding.

G-Statistics and Related Base Level Stats by Locus as Estimated Over Pops

Table 6: Genetic differentiation between the Group A and Group B samples. Fixation index (F_{ST}), Nei's genetic differentiation coefficient (G_{ST}), Hedrick's standardized differentiation (G''_{ST}), and Jost's differentiation index (D_{est}) indicate low but statistically significant population structuring. Confidence intervals were estimated by bootstrapping over loci.

Metric	Value	95% CI	p-value	Interpretation
F_{ST}	0.031	0.023–0.041	0.028	Very low differentiation
G_{ST}	0.012	0.002–0.023	0.028	Minimal population structuring
G''_{ST}	0.063	0.009–0.119	0.028	Low allele differentiation
D_{est}	0.040	0.006–0.081	0.028	Limited allele frequency divergence

Genetic differentiation between the two regional groupings was low but statistically significant. The overall F_{ST} value (0.031) indicates minimal genetic structuring, suggesting substantial shared ancestry and historical or ongoing gene flow between the northern (Group A) and western (Group B) regions. G_{st} (0.012) produced a similarly low estimate of differentiation, while Hedrick's standardized measure (G''_{ST} = 0.063) and Jost's D (D_{est} = 0.040) further support the presence of only limited allele frequency divergence between groups. Although differentiation remained statistically significant (p = 0.028), the low magnitude of these values indicates that the populations are not strongly genetically isolated. Instead, the results support weak regional structuring within a broadly connected population, likely maintained through historical connectivity, dispersal, translocations, and reserve-based management practices. Overall, the differentiation metrics indicate that most genetic variation is shared across regions rather than partitioned between them, consistent with ongoing connectivity and admixture among the sampled cheetahs.

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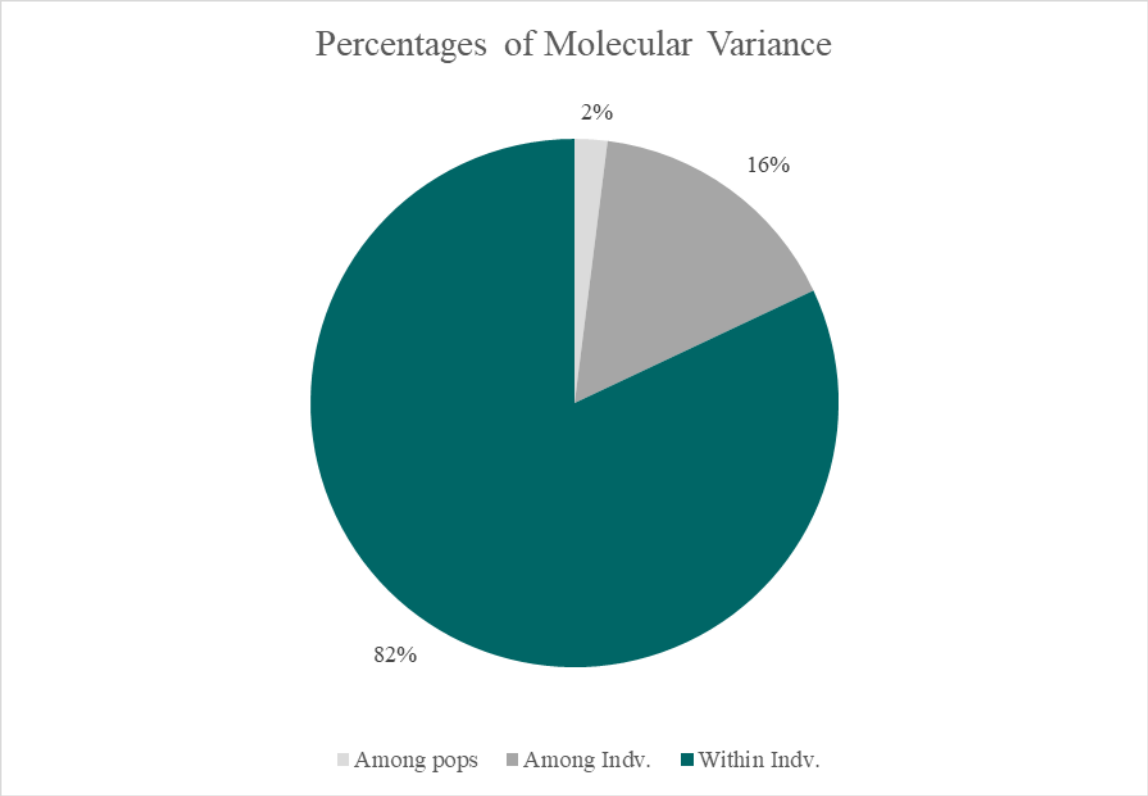

Figure 4: Percentage of molecular variance partitioned within and among regional cheetah groupings based on AMOVA.

Analysis of molecular variance (AMOVA) revealed that most genetic variation occurred within individuals (82%), while a smaller proportion was attributed to differences among individuals within populations (16%). Only a very small proportion of the total genetic variation was explained by differences between the northern (Group A) and western (Group B) regional groupings (2%). The overall PhiPT value indicated low genetic structuring between regions, supporting the presence of substantial shared genetic variation and ongoing connectivity among the sampled cheetahs. Although the among-population component remained statistically significant, the low proportion of regional variance suggests that the populations are not strongly genetically isolated and instead form part of a broadly connected and genetically cohesive population.

3.3 Comparative interpretation across populations

Across the two regional groupings analysed, genetic diversity levels were broadly comparable, although subtle differences in population structure were evident. The Group A samples exhibited slightly higher allelic diversity and expected heterozygosity, indicating a broader genetic base within the northern sampling region. However, this group also showed a heterozygote deficit and several deviations from Hardy-Weinberg equilibrium, suggesting the presence of fine-scale population substructure, localized relatedness, or restricted gene flow among subgroups. In contrast, the Group B samples displayed moderate genetic diversity with observed heterozygosity slightly exceeding expected heterozygosity and a mildly negative inbreeding coefficient (FIS = −0.034), indicating little evidence of widespread inbreeding and supporting the interpretation of a relatively well-mixed and genetically stable population. Although several loci deviated from Hardy-Weinberg equilibrium, overall patterns suggest only limited fine-scale structuring within this regional grouping. However, interpretation of the Group B dataset should be approached with some caution due to the relatively small sample size (n = 11), which may limit the ability to fully capture the genetic diversity and structure present within the broader population.

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Measures of genetic differentiation indicated low but statistically significant structuring between the regions (F_{ST} = 0.031; G_{ST} = 0.012), while standardized and allelic differentiation indices (G''_{ST} = 0.063; D_{est} = 0.040) revealed only limited allele frequency divergence between groups. Analysis of molecular variance further supported these findings, with most genetic variation occurring within individuals (82%) and among individuals within populations (16%), while only 2% of the total variation was attributed to differences between regional groupings. Together, these results indicate that the sampled cheetahs form a broadly connected and genetically cohesive population with only weak regional structuring. While localized relatedness and subtle allele-frequency differences are present, the overall patterns support substantial shared ancestry and ongoing connectivity across regions, highlighting the importance of maintaining gene flow and landscape connectivity in future conservation and management strategies.

3.4 Individual-level relatedness and inbreeding within populations

Pairwise relatedness (see Table 7) and inbreeding coefficients (see Table 8) were estimated using the Trio Maximum Likelihood (TrioML) and Dyad Maximum Likelihood (DyadML) estimators implemented in *COANCESTRY*. These estimators were selected based on their suitability for populations with low to moderate genetic diversity, uneven allele frequencies, and limited sample sizes, all of which are characteristic of the cheetah datasets. Unlike moment-based estimators, TrioML and DyadML are likelihood-based methods that estimate relatedness and inbreeding by maximising the probability of the observed genotypes given population allele frequencies, thereby reducing bias associated with allele frequency estimation error. The TrioML estimator incorporates information from a third individual to improve estimation accuracy, while DyadML relies on pairwise (comparing one individual to another) genotype likelihoods. Concordance between the two estimators therefore provides increased confidence in the robustness of the relatedness estimates.

Individual inbreeding coefficients were estimated using the Ritland and Lynch & Ritland estimators implemented in COANCESTRY. These estimators infer inbreeding from deviations in heterozygosity relative to population allele frequencies and are well suited to microsatellite datasets with limited sample sizes and uneven allele frequencies. Because TrioML and DyadML are designed to estimate relatedness rather than individual inbreeding, Ritland and Lynch & Ritland estimates were used specifically to assess inbreeding levels within each group.

Table 7: Relatedness summary statistics across Group A and Group B datasets as calculated in COANCESTRY.

	Group A	**Group B**
TrioML	0.046 (*Var: 0.011*)	0.051 (*Var: 0.010*)
DyadML	0.052 (Var: 0.013)	0.060 (Var: 0.014)

Table 8: Inbreeding coefficient summary across Group A and Group B datasets as calculated in COANCESTRY.

	Group A	**Group B**
Ritland	0.122 (*Var: 0.012*)	-0.030 (*Var: 0.007)*
LynchRd	0.144 (*Var: 0.021*)	-0.050 (*Var: 0.007*)

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Mean relatedness values were low in both regional groupings, indicating that most sampled individuals were not closely related. Group A exhibited low relatedness estimates (TrioML = 0.046; DyadML = 0.052), consistent with a broader genetic base and substantial mixing among individuals. However, inbreeding coefficients for this group were moderately elevated (Ritland = 0.122; Lynch & Ritland = 0.144), suggesting some mating among related individuals or the presence of fine-scale population substructure within the northern sampling region. These findings are consistent with the heterozygote deficit and deviations from Hardy-Weinberg equilibrium observed in Group A.

Group B displayed similarly low relatedness estimates (TrioML = 0.051; DyadML = 0.060), indicating limited overall kinship among sampled individuals and supporting the interpretation of a relatively well-mixed population. Inbreeding estimates for this group were negative (Ritland = −0.030; Lynch & Ritland = −0.050), indicating little evidence of inbreeding and suggesting a mild excess of heterozygosity relative to expectations under random mating. Together, these patterns suggest that while both regional groupings form part of a broadly connected population, signs of localized mating or subtle substructure are more evident within Group A, whereas Group B appears genetically stable with limited evidence of close-kin breeding. However, interpretation of Group B should be approached with some caution due to the smaller sample size available for this regional grouping.

Table 9: Inbreeding coefficients for individuals of the Group A dataset as calculated in COANCESTRY.

Individual	**Ritland**	**Lynch**
FRP0002_U60523	0.3414	0.5746
FRP0004_U60525	0.1963	0.3408
FRP0005_U60526	0.0996	0.0866
FRP0008_U60529	0.1222	0.1771
FRP0009_U60530	0.0418	0.0441
FRP0014_U60535	0.0918	0.1032
FRP0015_U60536	-0.0123	-0.0085
FRP0016_U60537	0.1187	0.1444
FRP0018_U60539	0.1253	0.1260
FRP0019_U60540	0.0803	0.0357
FRP0021_U60542	0.1465	0.1714
FRP0022_U60543	0.3420	0.3706
FRP0023_U60544	0.1573	0.2051
FRP0024_U60545	0.0674	0.1055
FRP0025_U60546	-0.0483	-0.0466
FRP0026_U60547	0.0822	0.0652
FRP0028_U60549	-0.0435	-0.0416
FRP0041_U60561	0.1622	0.1925
FRP0042_U60562	0.1943	0.1292
FRP0043_U60563	0.3348	0.2955
900182002321430_U60654	-0.0092	-0.0112
993021200013922_U60655	0.0825	0.1001

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Table 10: Inbreeding coefficients for individuals of the Group B dataset as calculated in COANCESTRY.

Individual	Ritland	LynchRd
FRP0001_U60522	-0.0589	-0.0831
FRP0007_U60528	-0.0386	-0.0458
FRP0031_U60552	-0.0084	-0.0117
FRP0033_U60554	-0.1636	-0.2393
FRP0034_U60555	-0.0478	-0.0451
FRP0039_U60560	-0.0872	-0.1361
FRCCCHEETAHSKIN_U606	0.0247	0.0191
FRP0010_U60531	-0.0517	-0.0516
FRP0013_U60534	0.0087	0.0129
FRP0044_U60564	-0.0865	-0.0624
FRP0037_U60558	0.1904	0.0949

3.5 Integrated interpretation of relatedness and inbreeding

Although relatedness and inbreeding are closely linked, they capture different aspects of population genetic structure. Relatedness reflects the average degree of kinship among individuals within a population, whereas inbreeding measures the probability that an individual has inherited identical alleles from related parents. As a result, populations may exhibit elevated relatedness without strong inbreeding if close relatives are not mating, or elevated inbreeding without uniformly high relatedness if related matings occur within particular subgroups.

This distinction is evident in the present datasets (see Figure 5A and 5B). Both regional groupings exhibited low mean relatedness, indicating that most sampled individuals were not closely related overall. However, Group A displayed moderately elevated inbreeding coefficients despite relatively low relatedness estimates, suggesting the presence of localized mating structure, fine-scale subpopulation subdivision, or restricted gene flow among subgroups within the northern sampling region. These findings are consistent with the heterozygote deficit and Hardy-Weinberg deviations observed in this group.

In contrast, Group B exhibited similarly low relatedness estimates together with negative inbreeding coefficients, indicating little evidence of close-kin mating and supporting the interpretation of a relatively well-mixed population. The negative inbreeding values further suggest a mild excess of heterozygosity relative to expectations under random mating. Together, these patterns indicate that while subtle regional structuring exists, the sampled cheetahs form a broadly connected population in which inbreeding risk appears more localized within Group A rather than widespread across the population. However, interpretation of Group B should be approached cautiously due to the smaller sample size available for this regional grouping.

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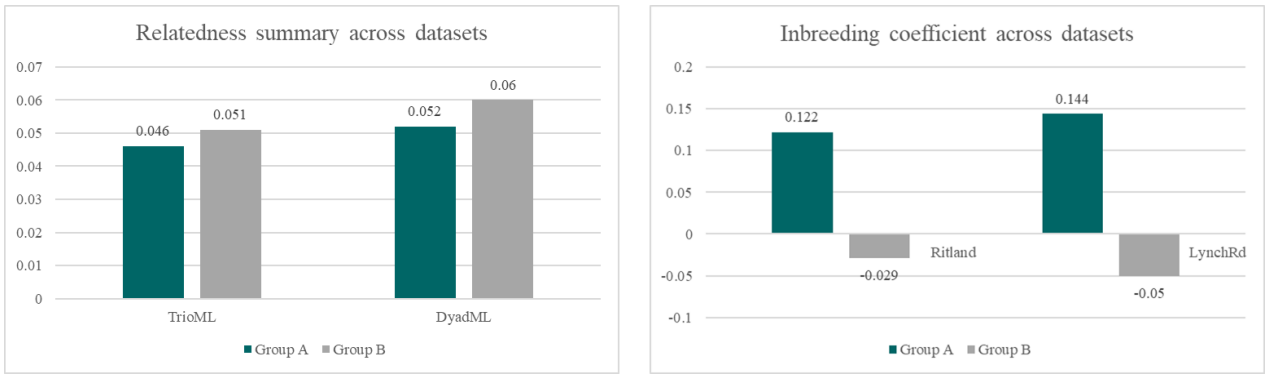

Figure 5A): Relatedness summary across Group A and Group B datasets. 5B): Inbreeding coefficient summary across Group A and Group B datasets as calculated in COANCESTRY.

3.6 Relationships among the two datasets:

Across the datasets, pairwise population Nei's Genetic Distance (Nei D) and Identity (Nei I), as well as F_{ST} values and estimated gene flow (N_m), were calculated to assess regional connectivity and genetic differentiation. Genetic differentiation between Group A and Group B was low (F_{ST} = 0.031), consistent with high estimated gene flow (N_m = 7.736) and strong genetic similarity based on Nei's measures (D = 0.046; I = 0.955). The low Nei's genetic distance and high genetic identity indicate substantial shared ancestry and limited divergence between the regional groupings. Collectively, these results support the presence of ongoing or recent connectivity between regions and suggest only weak regional structuring within a broadly connected population rather than strong genetic isolation. Although N_m estimates should be interpreted cautiously due to their dependence on model assumptions and equilibrium conditions, the concordant patterns observed across F_{ST}, gene flow, and Nei's distance metrics provide strong support for substantial connectivity among the sampled cheetahs.

To further illustrate relationships among datasets, a dendrogram (see Figure 6) was constructed using genetic distances between individuals. Unlike STRUCTURE, which assigns individuals to genetic clusters, the dendrogram summarises overall genetic relationships among individuals based on distance metrics. This dendrogram provides a visual representation of genetic similarity among the sampled cheetahs based on their DNA profiles. Each label represents an individual animal, and the branching structure illustrates how genetically similar or different individuals are from one another. The horizontal branch lengths reflect genetic distance: individuals that join together on short branches are genetically more similar, while those that connect only at longer branch lengths are more genetically distinct. Importantly, this diagram does not represent a family tree or direct ancestry, but rather the relative genetic similarity among individuals within the population.

Several tight clusters are visible where individuals join at very short branch lengths. These groupings indicate high genetic similarity and are consistent with close familial relationships. The presence of these clusters confirms the relatedness analyses and demonstrates that the genetic methods are accurately detecting family relationships within the population. Individuals from both geographic sampling regions are distributed throughout the dendrogram rather than forming distinct regional blocks. If the regions were genetically isolated, clear separation between Group A and Group B would be expected.

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Instead, individuals from different regions cluster together based on genetic similarity rather than geographic origin, indicating substantial shared ancestry and ongoing connectivity across the sampled landscape.

Most individuals connect to the broader tree at moderate branch lengths rather than extremely long ones, indicating that the population shares a common genetic background and has not diverged into highly distinct genetic lineages. This pattern is typical of populations that remain connected through movement, dispersal, or historical gene flow. Furthermore, the absence of deep hierarchical splits dividing the tree into major subdivisions indicates limited genetic differentiation and suggests that the population functions as a single, interconnected genetic unit. Rather than representing discrete regional populations, the dendrogram illustrates localized kin clusters embedded within a broader cohesive population structure.

The dendrogram visually reinforces the results obtained from the statistical analyses. Low genetic differentiation (FST = 0.031), low genetic distance (Nei's D = 0.046), and high genetic identity (Nei's I = 0.955) indicate minimal genetic separation between the regional groupings. Similarly, the high estimated gene flow (Nm = 7.736) suggests substantial movement and interbreeding across the landscape, supporting the interpretation of ongoing connectivity among sampled cheetahs. Analysis of molecular variance further demonstrated that only a small proportion of total genetic variation was attributable to differences between populations, while the majority of variation occurred within individuals and within populations, further supporting the absence of strong regional subdivision. Relatedness analyses identified localized kin clusters without evidence of widespread inbreeding, indicating that while relatives are present within the population, breeding patterns are not dominated by close-kin matings.

Taken together, these findings indicate that the sampled cheetahs form a genetically connected population with localized family groups but little evidence of strong regional isolation. Individuals from different areas share ancestry and contribute to a common gene pool, while continued gene flow appears to maintain connectivity across regions. This pattern reflects a positive genetic structure in which connectivity helps preserve genetic diversity and reduces the risks associated with isolation and inbreeding. From a conservation and management perspective, the dendrogram suggests that the population is not genetically fragmented and that maintaining landscape connectivity and opportunities for movement will remain important for preserving long-term genetic health. Overall, the dendrogram illustrates a cohesive population structure in which localized kin relationships exist within a broadly interconnected genetic network.

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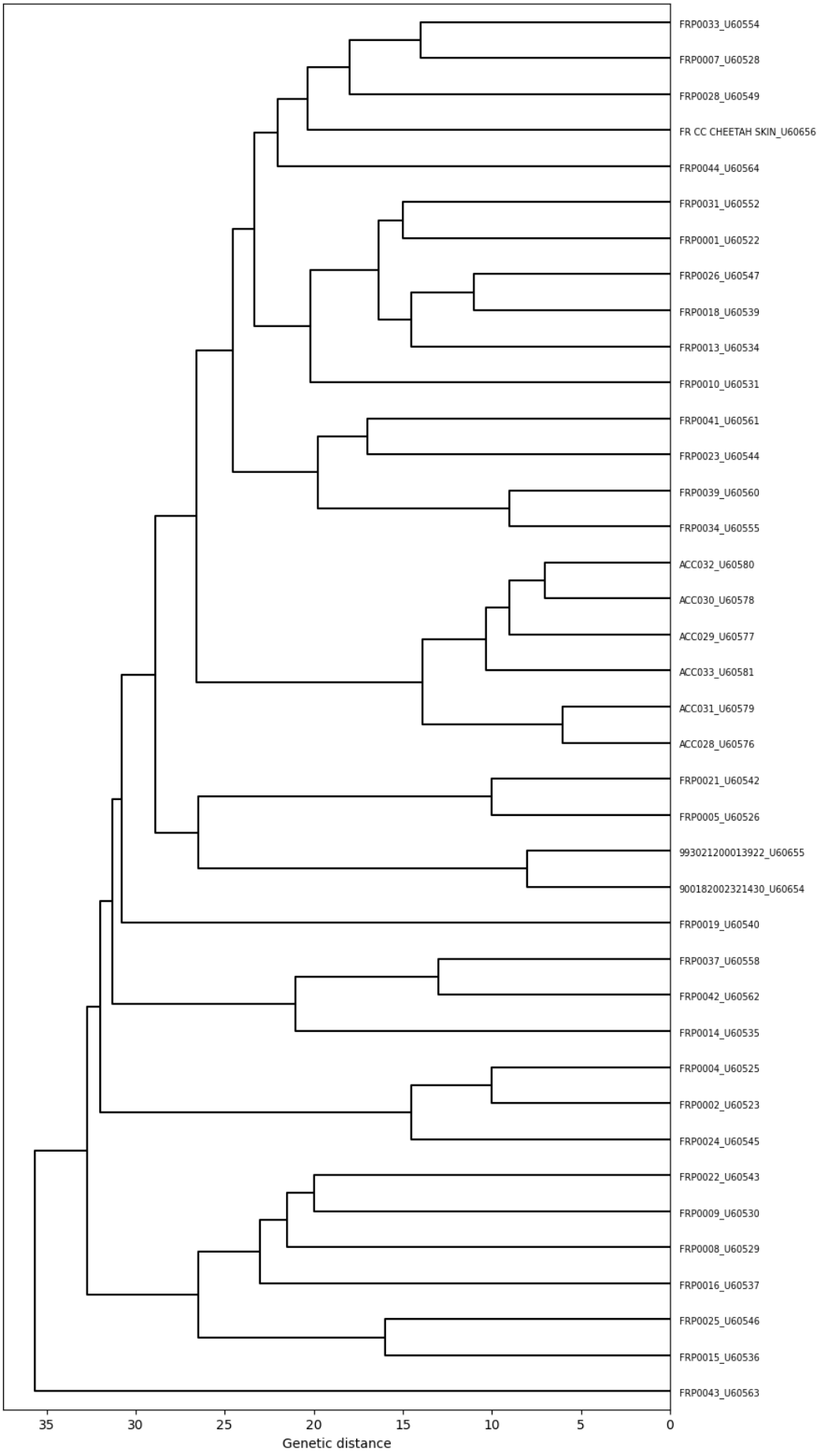

Figure 6: Dendrogram tree based on genotypic distances, showing relative genetic similarity and divergence among cheetah datasets.

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3.7 Bayesian STRUCTURE Analysis findings:

A Bayesian Admixture STRUCTURE analyses was performed on the Group A and Group B datasets (see Figure 7).

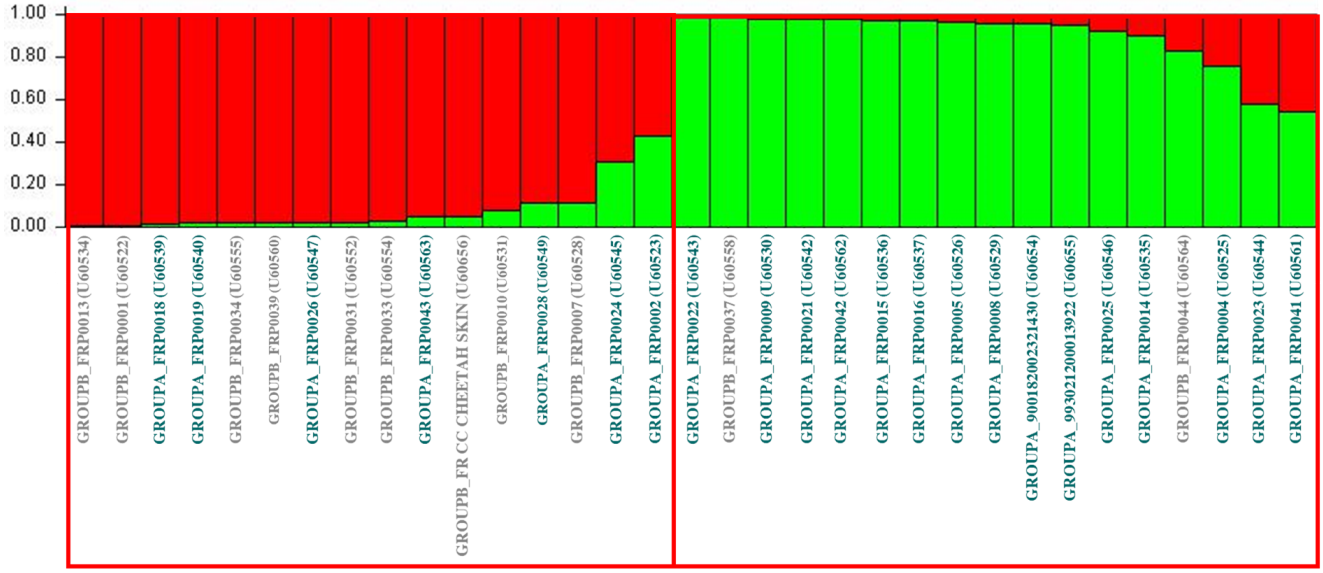

Figure 7A): Population Admixture across datasets with clustering = 2 groups as estimated in STRUCTURE.

Datasets were analysed using STRUCTURE with samples grouped into two regional units (Group A and Group B). Bayesian clustering analysis supported the presence of two genetic clusters (K = 2), although these clusters did not correspond exclusively to geographic sampling regions. Cluster 1 consisted of a mixed grouping containing approximately 44% Group A individuals and 56% Group B individuals, while Cluster 2 was predominantly composed of Group A samples (approximately 88%), with only limited representation from Group B (approximately 12%). The presence of individuals from both regional groupings within each cluster indicates substantial admixture and shared ancestry across regions rather than strong geographic separation.

Although one cluster showed a greater representation of Group A individuals, the overall clustering pattern did not reveal clear genetic isolation between the regional groupings. Instead, the STRUCTURE results suggest subtle regional allele-frequency differences superimposed on a broadly connected population. The mixed ancestry observed across clusters indicates ongoing or historical gene flow between regions and supports the interpretation of incomplete population subdivision rather than the presence of discrete genetic populations or management units.

These findings are consistent with the broader genetic analyses. Low genetic differentiation between regions (F_{ST} = 0.031), low Nei's genetic distance (D = 0.046), and high genetic identity (I = 0.955) indicate minimal population divergence, while high estimated gene flow (N_m = 7.736) suggests substantial movement and interbreeding across the landscape. Analysis of molecular variance further demonstrated that only a small proportion of genetic variation was attributable to differences between populations, supporting the conclusion that the sampled cheetahs form a largely shared gene pool. The weak

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clustering observed in STRUCTURE is therefore best interpreted as subtle regional structuring within a connected population rather than evidence of strongly differentiated genetic groups.

Overall, the STRUCTURE analysis reinforces the conclusion that the sampled cheetahs represent a genetically cohesive population characterized by substantial shared ancestry, ongoing connectivity, and only limited regional structuring. While weak allele-frequency differences between regions are detectable, the extensive admixture observed across clusters suggests that gene flow remains sufficient to prevent strong genetic isolation. Maintaining this connectivity will likely be important for preserving genetic diversity and supporting long-term population viability.

4. Concluding remarks: Integrated interpretation across diversity, genetic distance, gene flow, relatedness, inbreeding, and STRUCTURE

When considered together, measures of genetic diversity, genetic distance, gene flow, relatedness, inbreeding, and Bayesian clustering analyses reveal a coherent pattern of substantial connectivity with only weak regional structuring among the sampled cheetahs. Pairwise differentiation metrics and gene flow estimates indicate that the northern sampling region (Group A: Limpopo and Botswana) and the western region (Group B: North West and Northern Cape) form a broadly connected genetic network characterized by substantial shared ancestry. Low genetic differentiation (F_{ST} = 0.031), low Nei's genetic distance (0.046), high genetic identity (0.955), and high inferred gene flow (N_m = 7.736) collectively indicate limited regional divergence and ongoing or recent connectivity between regions. Analysis of molecular variance further supports this pattern, demonstrating that only a small proportion of total genetic variation is attributable to differences between regional groupings, while the majority of variation occurs within individuals and within populations.

Despite this overall connectivity, individual-level analyses revealed subtle fine-scale structuring within the broader population. The dendrogram and relatedness analyses identified localized clusters of genetically similar individuals embedded within an otherwise interconnected population structure. However, mean relatedness values remained low in both regional groupings, indicating that most sampled individuals were not closely related overall. Group A exhibited moderately elevated inbreeding coefficients despite relatively low relatedness estimates, suggesting localized mating structure, subtle subpopulation subdivision, or restricted gene flow among subgroups within the northern sampling region. In contrast, Group B displayed similarly low relatedness together with negative inbreeding coefficients, indicating little evidence of widespread inbreeding and supporting the interpretation of a relatively well-mixed population with mild heterozygote excess.

Bayesian clustering analyses further supported these findings. STRUCTURE analysis identified two genetic clusters (K = 2), although these clusters did not correspond exclusively to geographic sampling regions. Both clusters contained individuals from Group A and Group B, with one cluster showing a greater representation of Group A individuals while the second contained a more balanced mixture of samples from both regions. The extensive admixture observed across

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clusters indicates substantial shared ancestry and ongoing connectivity rather than strong geographic isolation. The weak clustering patterns observed are therefore best interpreted as subtle regional allele-frequency differences within a connected population rather than evidence of discrete genetic populations or management units.

The concordance among differentiation metrics, AMOVA results, relatedness analyses, dendrogram structure, and Bayesian clustering strongly supports the conclusion that the sampled cheetahs form a genetically cohesive population with only limited regional structuring. While localized relatedness and subtle allele-frequency differences are present, there is little evidence of strong fragmentation or widespread close-kin breeding. Instead, the data suggest that gene flow and connectivity across the landscape remain sufficient to maintain substantial genetic similarity among regions.

From a conservation and management perspective, these findings indicate that the population is not strongly genetically isolated and that maintaining landscape connectivity and opportunities for dispersal will remain important for preserving long-term genetic diversity and reducing future inbreeding risk. Continued genetic monitoring is advisable, particularly within smaller or potentially semi-isolated subgroups, to ensure that localized structuring does not intensify over time. The relatively small sample size available for Group B should also be considered when interpreting regional patterns, as additional sampling may further refine estimates of diversity and population structure.

Taken together, this study provides a robust genetic baseline for assessing the current status of regional cheetah groups and informing future conservation and management strategies. Although weak regional structuring is detectable, the overall genetic patterns support the presence of a broadly connected and genetically cohesive population characterized by substantial shared ancestry and ongoing gene flow. Establishing this baseline enables informed, proactive conservation planning and provides a framework for evaluating future changes in genetic diversity, connectivity, and population structure over time.

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